"ARGYROPHILIC NUCLEOLAR ORGANIZER REGIONS (AgNORs) AS A PROLIFERATIVE MARKER INBENIGN, PREMALIGNANT AND MALIGNANT PROSTATIC LESIONS"

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M.D. DEGREE

In

PATHOLOGY – BRANCH III

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DR. M.G.R. MEDICAL UNIVERSITY

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DECLARATION

I hereby declare that the dissertation entitled ARGYROPHILIC NUCLEOLAR ORGANIZERREGIONS (AgNORs) AS A PROLIFERATIVE MARKER IN BENIGN, PREMALIGNANT AND MALIGNANT PROSTATIC LESIONSwas done by me in the Department Of Pathology at Coimbatore Medical College, Coimbatore during the period from June 2016- May2017under the guidance of Dr.C.LALITHA, M.D., Professor and Head Of The Department, Coimbatore Medical College, Coimbatore.

This dissertation is submitted to the TamilnaduDr.M.G.R Medical University, Chennai towards the partial fulfillment of the requirement for the award of M.D., Degree in Pathology.

I have not submitted this dissertation on any previous occasion to any university for the award of any degree.

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CERTIFICATE

This is to certify that dissertation entitled ARGYROPHILIC NUCLEOLAR ORGANIZER REGIONS (AgNORs) AS A PROLIFERATIVE MARKER IN BENIGN, PREMALIGNANT AND MALIGNANT PROSTATIC LESIONS is a record of bonafide work done by Dr.T.KANIMOZHI, Postgraduate Student in the Department of Pathology, Coimbatore Medical College and Hospital, Coimbatore under the supervision and guidance of Dr. C.LALITHA, M.D., Professor and Head, Department of Pathology, Coimbatore Medical College and Hospital, Coimbatore in partial fulfillment of the regulations of the Tamilnadu Dr.M.G.R. Medical University, Chennai towards the award of M.D. degree (Branch III) in Pathology.

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CERTIFICATE – II

This is to certify that this dissertation work titled "Argyrophilic Nucleolar Organizer Regions (AgNORs) as A Proliferative Marker In Benign, Premalignant and Malignant Prostatic Lesions" of the candidate Dr. KANIMOZHI.Twith registration Number 201513252 for the award of M.D in the branch of Pathology. I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows 1% (one) percentage of plagiarism in the dissertation.

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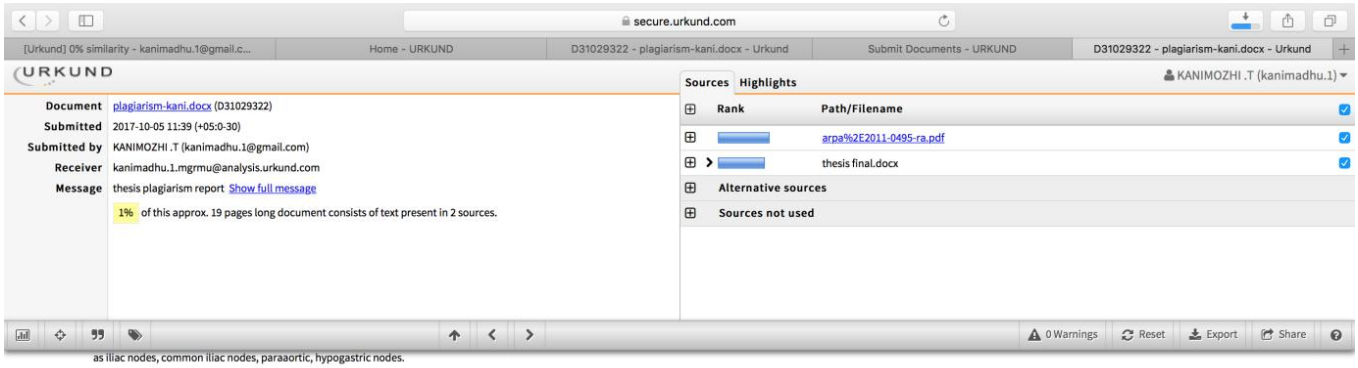

as iliac nodes, common iliac nodes, paraaortic, hypogastric nodes.

It can metastasise to bone, liver, thorax, brain, GIT, retroperitoneum, kidney

and adrenals.

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OF PROSTATIC CARCINOMA:

'TX - Primary tumor cannot be assessed.

T0 - No evidence of primary

tumor

T1 - Clinically inapparent tumor neither palpable nor visible by imaging T1a - Tumor incidental histologic finding in 5% or less of tissue resected

T1b - Tumor incidental histologic finding in more than -5% of tissue resected

T1c - Tumor identified by needle biopsy (e.g., because of elevated PSA).

Includes tumors found in both lobes by needle biopsy.

pT2 - Tumor confined within the prostate

pT2a- Unilateral, one half of one side or less.

pT2b- Unilateral, involving more than one half of one side but not both sides.

pT2c- Bilateral (B/L) disease

pT3 - Extraprostatic extension.

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CONTENTS

S.NO	PARTICULARS	PAGE NO
1	INTRODUCTION	1
2	AIM OF THE STUDY	3
3	OBJECTIVE OF THE STUDY	4
4	REVIEW OF LITERATURE	5
5	MATERIALS AND METHODS	41
6	OBSERVATION AND RESULTS	45
7	DISCUSSION	67
8	SUMMARY	74
9	CONCLUSION	76
10	ANNEXURES a) ANNEXURE -1 PROFORMA b) ANNEXURE –II HAEMATOXYLIN AND EOSIN PROCEDURE	77
11	BIBLIOGRAPHY	81

LIST OF ABBREVIATIONS

1.	NOR	-	Nucelolar Organizer Regions
2.	Ag	-	Silver
3.	BPH	-	Benign Prostatic Hyperplasia
4.	PIN	-	Prostatic Intraepithelial Neoplasia
5.	PC	-	Prostatic Carcinoma.
6.	PSA	-	Prostate Specific Antigen.
7.	TURP	-	Transurethral resection of prostate
8.	IHC	-	Immunohistochemistry

LIST OF TABLES

S.NO	TABLE	PAGE NO.
1.	**Mean age of the study**	45
2.	**Age distribution of prostatic lesions**	46
3.	**Type of Biopsies in prostatic lesions**	47
4.	**Diagnosis of various prostatic lesions**	48
5.	**Age wise distribution of various prostatic lesions**	49
6.	**Mean AgNOR COUNTof various prostatic lesions**	50
7.	**Gleason grade in PC Patients**	51
8.	**Mean AgNOR COUNTof Gleason grade in PC Patients**	52
9.	**Mean AgNOR COUNTof various prostatic lesions (within the groups) using ANOVA**	53

LIST OF CHARTS

S.NO	CHARTS	PAGE NO.
1.	**Age distribution of prostatic lesions**	46
2.	**Type of Biopsies in prostatic lesions**	47
3.	**Diagnosis of various prostatic lesions**	48
4.	**Age wise distribution of various prostatic lesions**	49
5.	**Mean AgNOR COUNTof various prostatic lesions**	50
6.	**Gleason grade in PC Patients**	51
7.	**Mean AgNOR COUNTof Gleason grade in PC Patients**	52
8.	**Mean AgNOR COUNTof various prostatic leisons (within the groups) using ANOVA**	54

COLOUR PLATES

S.NO	FIGURES
1	Benign Prostatic Hyperplasia- H&E Stain 10x magnification
2	Benign Prostatic Hyperplasia-AgNOR stain.
3.	Benign Prostatic Hyperplasia - H&E stain 40x magnification
4.	AgNOR dots in Benign Prostatic Hyperplasia
5.	Benign Prostatic Hyperplasia –H&E stain 40 x power.
6.	Benign Prostatic Hyperplasia- Large, Regular, round AgNOR dots.
7.	Benign Prostatic Hyperplasia H&E stain. 40X magnification.
8.	AgNOR dots in Benign Prostatic Hyperplasia- Large, Regular, round and homogenously stained.
9.	PROSTATIC ADENOCARCINOMA (H&E STAIN) 10X magnification.
10.	AgNOR dots (in adenocarcinoma) Gleason Grade7
11.	Prostatic adenocarcinoma- H&E stain.40x magnification.
12.	Prostatic adenocarcinoma : Gleason Grade 9
13.	Prostatic Adenocarcinoma : Gleason Grade 8
14.	Prostatic Adenocarcinoma: Gleason Grade 8
15.	Prostatic Adenocarcinoma : Gleason Grade 9

16.	Prostatic Intraepithelial Neoplasia H&E stain 40x.
17.	Prostatic Intraepithelial Neoplasia- Increased number of AgNOR dots.
18.	Prostatic Intraepithelial Neoplasia. H&E stain 10x
19.	mAgNOR count of 2.8 in Prostatic Intraepithelial Neoplasia.

INTRODUCTION

Prostatic carcinoma became as one of the most common malignancy among men[1]. Its incidence has increased due to older age, familial cause, change in lifestyle factors such as high calorie intake and lack of exercise. Prostate cancer can spread to bones, nodes and other organs.

Benign prostatic hyperplasia can produce difficulty in urination, haematuria or pelvic pain.Early diagnosis and prompt treatment is necessary to improve the outcome/ prognosis of the patient.Serum prostatic specific antigen (PSA) levels, histologic grade are used in the diagnosis of various prostatic lesions.

Though routine H&E (Haematoxylin and eosin) can differentiate benign, premalignant and malignant lesions ,there are certain limitations[2] such as prostatic needle biopsy itself presents problems like small amount of tissue is provided for microscopic examination and also the difficulties in distinguishing cancer from its benign mimickers[3] such as atrophy, basal cell hyperplasia, inflammatory atypia and small crowded glands.

There is no single criteria to diagnose prostate cancer. The combination of architectural and cytological features can diagnose prostate cancer[4].

Wrong diagnosis leads to serious problems such as unwanted exposure to radiation induced side effects, unnecessary prostatectomies due to false positive diagnosis.

Even false negative diagnosis can lead to missing of early diagnosis and results in delaying of starting the treatment.Hence proper definitive diagnosis is necessary to benefit the patients.The present AgNOR study highlights the differentiation of benign, premalignant and malignant lesions of the prostate[5].

Significant increase in AgNOR count is noted in malignancy and low AgNOR count is seen in benign prostatic hyperplasia[6].Also, to assess the proliferative activity of the cell and for prognostic point of view, among various proliferative markers, AgNOR is used in the present study.

Staining of the nucleolar organizing regions by silver compound[7] has gained popularity for its simplicity, easy and cost effective procedure compared to other proliferative markers.

AIM

The present study is aimed at evaluating the importance of AgNOR in differentiating benign, premalignant and malignant lesion of the prostate.

OBJECTIVES OF THE STUDY

1. To establish the significance of Nucleolar organizer regions in various prostatic lesions such as benign, premalignant and malignant prostaticlesions[8].
2. To correlate the AgNOR count with histological grade in prostatic Carcinoma[9].
3. To evaluate AgNOR study as a diagnostic and prognostic marker in prostate cancer.

REVIEW OF LITERATURE

ANATOMY OF PROSTATE GLAND:

Prostate is an unpaired accessory structure of the male reproductive system that surrounds the urethra in the pelvic cavity. It is found immediately inferiorto the bladder and anterior to the rectum. Prostate is an inverted rounded cone shaped structure which is continuous above with the neck of the bladder and its apex lies on the pelvic floor.

The average weight of the prostate gland weighs 30 to 40 gms. Prostatedevelops as 30 to 40 individual complex glands from the urethral epithelium into the surrounding wall of the urethra. Collectively, these glands develop into what is known as prostate. The ducts of these gland empty into the prostatic sinuses on the posterior aspect of the urethral lumen.

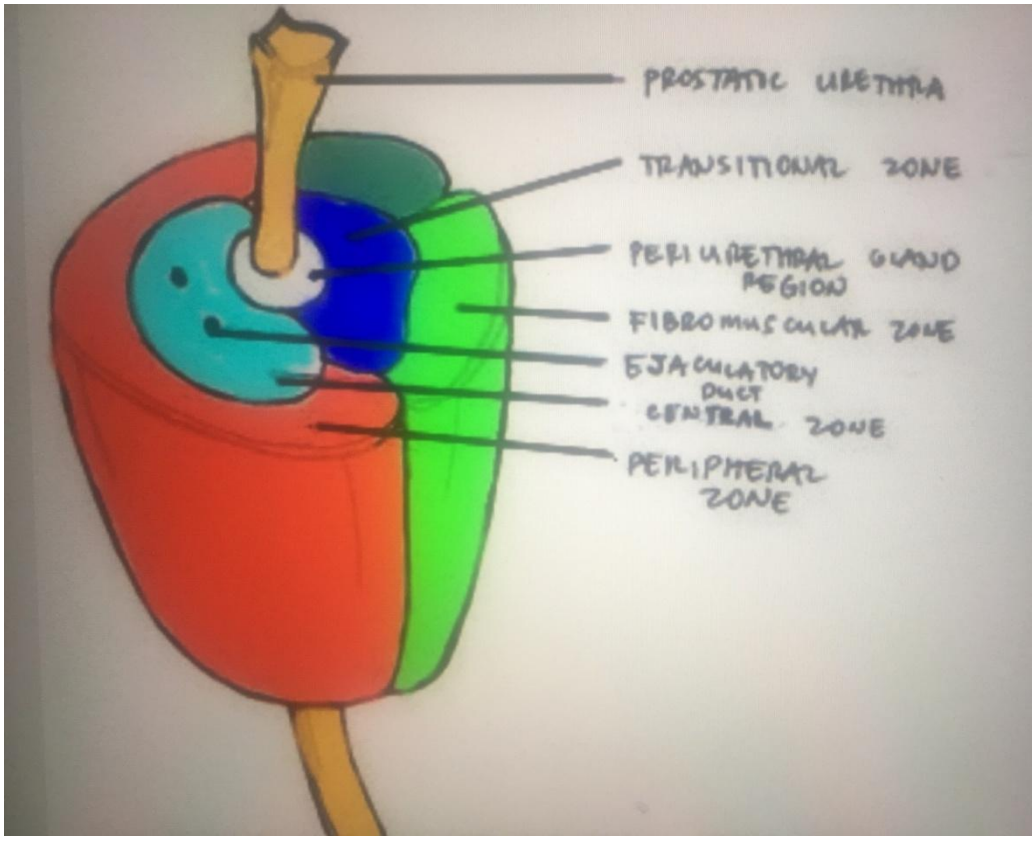
HISTOLOGY OF PROSTATE GLAND:

Prostate tissue is composed of glands surrounded by fibromuscular stroma.

These prostatic glands are lined by three types of cells: outer basal cells andinner acinar cells and neuroendocrine cells.Inner acinar/secretory layer is composed of single to pseudostratified columnar cells with nuclei placed perpendicular to the basement membrane having

moderate to abundant eosinophilic cytoplasm. Outer basal cell layer is characterized by flattened to low cuboidal cells with nuclei oriented parallel to the basement membrane. The secretory cells secrete PSA and PSAP.

The prostatic secretions are of neutral mucosubstances whereas in prostatic carcinomas, one can encounter both acid and neutral mucin substances. Corpora amylacea is a dense eosinophilic laminated secretion present within the lumen. The function of prostatic stroma containing smooth muscle cells is to squeeze the prostatic secretions when they are stimulated.



There are four zones in the prostate gland:

1. Transition zone: constitutes 5% of the prostate .This zone is seen surrounding the proximal urethra. Benign prostatic hyperplasia iscommon in this zone.
2. Central zone: constitutes 25% of the gland. This zone is seen
3. surrounding the ejaculatory ducts. Carcinoma developing in this zone behaves aggressively and involves seminal vesicles.
4. Peripheral zone: constitutes 70% of the gland. It is the subcapsular portion of the posterior aspect of the prostate gland surrounding the distal urethra. Majority of the prostatic carcinoma arises from this zone.
5. Anterior fibromuscular zone: constitutes 5% of the prostate gland. This zone is devoid of glandular components and is composed of only muscle and fibrous tissue.
6. Periurethral gland region: consists of tiny ducts and abortive acini within smooth muscle of pre prostatic sphincter around proximal prostatic urethra. It is the site of origin for primary urothelial carcinoma.

Sometimes, lobe classification is used for describing the anatomy of the prostate.

Anterior lobe- roughly corresponds to the part of transitional zone.

Posterior lobe – corresponds to the peripheral zone.

Median (or middle) lobe corresponds to the central zone.

Lateral lobe spans all zones.

BIOPSIES IN PROSTATE:

1.Needle biopsies.

Transrectal ultrasound guided biopsy (TRUS)

Transurethral biopsy

Transperineal prostate biopsy

2.Transurethral resection of prostate(TURP)

3. Suprapubic prostatectomy

4. Radical prostatectomy

- Sextant biopsy consists of 6 cores taken from bilateral base, midgland and apex.
- Extended biopsy consists of 10 to 12 cores which is taken from mid, lateral and peripheral zones in addition to sextant biopsy.
- Saturation biopsy consists of >20 cores. Biopsy is taken from transition zones in addition to extended biopsy[10].

It is done when there is persistent elevation of PSA levels despite several negative biopsies.It is very important to separate cores and know the site form where the biopsy was taken because, the central zone towards the base of the prostate mimicks high grade prostatic intraepithelial neoplasia which should be known to prevent over diagnosis and also to correlate the location of tumor in subsequent radical prostatectomy specimen when the tumor is extremely focal.

In transurethral resection of prostate, prostatic chips weighing <12 gms aresubmitted entirely. If it is >12gms , in addition to 12gms, 1 cassette for every 5gms are submitted.

Total serum PSA- Prostate specific antigen

It is a serine protease of the kallikrein family[11] secreted by the epithelial cells lining the acini and ducts. They are secreted into the duct lumen and helps to liquefy the coagulum and breakdown seminal clot through proteolysis and releases the spermatozoa. They reach the serum by diffusion across the basement membrane into the stroma and passes through the capillary basement membrane.

Normal serum PSA level- < 4ng/ml.

Serum PSA level of > 10ng/ml have increased risk for prostatic cancer of about 67 %[11].

Prostate specific antigen density:

It is calculated by dividing the total serum PSA levels by estimated gland volume which is assessed by trans rectal ultrasonography.

Prostate specific antigen velocity[6]:

It denotes the rate of change of prostate specific antigen per year. Atleast three serum PSA levels are done . Patients with prostate cancer have PSA velocity to be 0.75ng/ml/year.

IMMUNOHISTOCHEMISTRY[12]:

Secretory cells- are positive for PSA(Prostate specific antigen) , PSAP(Prostatespecific acid phosphatase).But can also coexist with vimentin and various keratins except High molecular weight keratins.

Myoepithelial cells- are positive for HMWK (High molecular weight keratin) –CK5 or CK5/6 and P63 (nuclear staining).

Neuroendocrine cells- are positive for Chromogranin A,B, secretogranin II, somatostatin, calcitonin, bombesin.

Alpha methyl CoA racemase- granular cytoplasmic staining is positive in prostatic carcinoma[13].

Stroma has androgen receptors.

WHO CLASSIFICATION OF TUMORS OF THE PROSTATE

Epithelial Tumors

- Glandular neoplasms
- Acinar neoplasms
  - Atrophic
  - Pseudohyperplastic
  - Microcystic
  - Foamy gland
  - Mucinous(colloid)
  - Signet ring like cell
  - Pleomorphic giant cell
  - Sarcomatoid
- Prostatic intraepithelial neoplasia
- Intraductal carcinoma
- Ductal adenocarcinoma
- Urothelial carcinoma
- Squamous neoplasms
- Basal cell carcinoma

Haematolymphoid tumors

Neuroendocrine tumors

Mesenchymal tumors

Metastatic tumors

BENIGN PROSTATIC HYPERPLASIA

It is the most common urologic disorder in men[14] and affects predominantly elderly men.

It elevates serum PSA levels and affects transitional zone of theprostate.

Patients presents with symptoms of obstruction such as difficulty in urination, and disturbances in urinary frequency, urgency and incontinence.

It involves proliferation of either pure stromal component or both stromal and epithelial components. The hallmark of prostatic hyperplasia isnodule formation[15]. These nodules are composed of glands lined by basal and secretory layer that forms small and large acini with papillary infoldings and projections and the stroma is made up of fibromuscular cells, blood vessels, few inflammatory cells in a myxoid or hyalinized matrix[14].

BASAL CELL HYPERPLASIA

Basal cell hyperplasia is more common in the transition zone. It is commonly seen with antiandrogen therapy.

It consists of proliferation of uniformly spaced small glands lined by multiple layers of basal cells oriented in both parallel and perpendicular direction to basement membrane with minimal cytoplasm, crowded nuclei and pinpointnucleoli[16]. Intraluminal eosinophilic secretions or calcifications and intracytoplasmic hyaline globules may be seen.

Basal cell markers like p63 will be positive[17]. AMACR is negative.

CLEAR CELL CRIBRIFORM HYPERPLASIA

It is an uncommon form of benign prostatic hyperplasia. Usually occurs in the transition zone.

It consists of many nodules which is composed of glands in cribriform pattern having lumens of varying sizes. These cells are small with bland uniform nuclei which lacks prominent nucleoli with clear cytoplasm. Basal cells are seen surrounding the glands.

Markers such as p63, HMWK highlights basal cells whereas AMACR is negative.

PROSTATIC INTRAEPITHELIAL NEOPLASIA:

It is a premalignant condition which is limited to the prostatic ducts and acini. It is common in the peripheral zone of the prostate. There is both architectural and cytological atypia.[18]

They are of two grades: Low grade and high grade.

High grade PIN have a darker gland appearance in low power.

There are four architectural patterns:

Tufting

Micropapillary

Cribriform

Flat.

Itconsists of pseudostratified secretory cells with cytological atypia which includes nucleomegaly, hyperchromasia, nuclear irregularity, coarse, clumped chromatin and prominent nucleoli[19]. These patients have increased risk for developing prostatic carcinoma.

An interesting point is to note here is, nuclei located more towards the basement membrane have more enlarged nuclei with prominent nucleoli and nuclei towards the centre of the gland have bland cytology[19].

Using double colour triple antibody which includes AMACR, HMWK, and p63, high grade PIN demonstrates patchy nuclear p63, HMWK cytoplasmic basal cell staining, and AMACR[20] red cytoplasmic staining.

PROSTATIC ADENOCARCINOMA

It is the most common cancer in men[1].

Risk factors includes age, and family history[21]. There is increase in serum prostate specific antigen levels.

Various environmental and genetic factors[22] are involved in development of prostatic carcinoma[23].

High levels of fat intake have increased risk whereas intake of fruits and vegetables have low risk for development of prostatic carcinoma[24].

Increased testosterone and dihydrotestosterone levels are more prone for carcinoma.

Exposure to herbicides and cadmium are also risk factors[25].

Most prostatic carcinoma is asymptomatic because of its location in peripheral Zone[26]. When the tumor is large enough to involve the transition zone, it can leads to urinary obstruction[27]. Some can involve the bladder and rectum and causes haematuria, rectal bleeding and obstruction.

Diagnosis depends on 4 factors which includes

Luminal

Architectural

Nuclear

Cytoplasmic features[5,28].

LUMINAL FEATURES

Wispy blue tinged mucin in the lumen of glands.

Crystalloids- rhomboid in shape

Comedo-necrosis

Granular eosinophilic secretions.

ARCHITECTURAL FEATURES

Well differentiated tumors are composed of well formed glands.

In poorly differentiated tumors, tumor cells are arranged in solid sheets.

NUCLEAR FEATURES

Nucleus is enlarged, hyperchromatic, pleomorphic with one or moreprominent nucleoli.

CYTOPLASMIC FEATURES:

The neoplastic cells have amphophilic cytoplasm (darker) compared to benign glands.

Mucinous fibroplasia is a collagenous nodules which is defined as eosinophilic hyaline material ,most often seen along with mucin in the lumen.

Glomerulations are intraluminal cellular proliferation and it is surrounded by acrescentic space resembling renal glomeruli. This is characteristic feature ofprostate carcinoma[29].

Perineural invasion is also a feature of prostate carcinoma.

VARIANTS OF PROSTATIC CARCINOMA[31,30]:

Ductal adenocarcinoma

Atrophic carcinoma

Pseudohyperplastic carcinoma

Foamy gland carcinoma

Mucinous carcinoma.

Signet ring carcinoma

Microcystic carcinoma

Pleomorphic giant cell carcinoma.

Small cell carcinoma

Sarcomatoid carcinoma

Urothelial carcinoma

Squamous cell carcinoma

Basaloid carcinoma

FOAMY GLAND VARIANT

It is an uncommon variant.

Neoplastic cells forming glands have abundant foamy appearing cytoplasm with nuclear cytoplasmic ratio such that nucleomegaly and prominent nucleoli are less prominent[32]. Some of the glands contains eosinophilic secretions. In many cases , it is associated with ordinary adenocarcinoma of prostate.

It is negative for basal cell markers and Positive for PSA and PSAP.

ATROPHIC VARIANT

Neoplastic glands have a distorted lumen and has a flat appearance.

The tumor show infiltrative pattern. Nuclear features such as nucleomegaly, nucleoli are prominent.

They occur in association with ordinary adenocarcinoma of prostate.

MUCINOUS / COLLOID ADENOCARCINOMA

It is a least common variant of prostatic adenocarcinoma.

Atleast 25% mucin pools should be present to name it as mucinous carcinoma[33].

Grossly, tumor nodules have mucinous and gelatinous texture. Single files or cribriform pattern of glands are seen in the mucin pools.

They are negative for basal cell markers and are positive for PSA and PSAP.

SIGNET RING CELL VARIANT

A rare variant of the prostatic adenocarcinoma.

It has a poor prognosis.

It is characterized by the presence of intracytoplasmic vacuoles that pushes the nucleus and giving the shape of a signet ring.

Atleast 25 to 50 % of the tumor should contain signet ring cells[34] for the diagnosis of this variant.

Tumor is predominantly composed of infiltrating single file pattern. Gleason score -5.

These are negative for basal cell markers and positive for PSA and PSAP.

LYMPHOEPITHELIAL LIKE VARIANT

It is a very rare variant.

There is a heavy lymphocytic infiltration admixed with syncytial pattern of malignant epithelial cells. Malignant cells have marked nuclear and cytoplasmic atypia.

Malignant cells are positive for PSA and cytokeratin.

Surrounding cells are predominantly T lymphocytes.A CD3 stain highlights the T cell population.

SARCOMATOID CARCINOMA

This tumor is composed of both malignant epithelial and mesenchymal components.

Nodal and distant metastasis are common.

PLEOMORPHIC GIANT CELL CARCINOMA

These neoplasms have the characteristic pleomorphic giant cells having bizarre nuclei with abundant eosinophilic cytoplasm and anaplastic features.

Presence of giant cells is a characteristic feature of this variant.

PROSTATIC INTRAEPITHELIAL LIKE DUCTAL ADENOCARCINOMA

This variant resembles PIN and consists of glands lined by pseudostratified columnar epithelial cells[35] . These glands appears crowded and have some architectural complexity such that intraluminal tufting is noted.

Nuclear atypia and pseudostratification is prominent.

Negative for basal cell markers (p63, HMWK) and positive for AMACR.

INTRADUCTAL CARCINOMA OF THE PROSTATE

It is characterized by the spread of the prostatic adenocarcinoma into prostatic ducts/ acini most often seen associated with invasive adenocarcinomatous components[36].

These patients have increased serum PSA levels[37].

It is seen in radical prostatectomy specimens.

Atypical malignant cells fill the prostatic ducts and acini[38].

These cells are arranged in solid, cribriform or in micropapillary patterns[39]. Basal cells are seen.

These malignant cells show nucleomegaly and have hyperchromatic, pleomorphic nuclei with prominent nucleoli.

Basal cells stain for p63, HMWK which indicates patchy or complete preservation of basal cell layer[40].

Neoplastic cells stain for AMACR.

PROSTATIC DUCTAL ADENOCARCINOMA:

It consists of ducts/acini filled with proliferation of columnar cells in cribriform or in papillary pattern.

It is also called as adenocarcinoma with endometrioid features (due to resemblance of endometrioid adenocarcinoma).

It has a poor prognosis and behaves in an aggressive manner compared to acinar adenocarcinoma.

Serum PSA levels are elevated.

It can occur either as pure form or mixed forms with acinar adenocarcinoma.

These cells are arranged in glandular, papillary or in cribriform pattern which have columnar, pseudostratified epithelium with amphophilic cytoplasm, elongated nuclei having prominent nucleoli. Mitosis and necrosis are also noted.

Basal cell markers like p63, HMWK are negative. AMACR is strongly positive.

SMALL CELL CARCINOMA:

It is a rare subtype of prostatic adenocarcinoma.

It is of high grade and have neuroendocrine differentiation.

It is seen in older males.

They have poor prognosis and presents at advanced stages.

The neoplastic cells are small with minimal cytoplasm having nuclear molding, fine chromatin. Rosette formation may be seen[41] and association with extensive tumor necrosis, apoptosis, karyrorrhexis , high mitotic rates.

They are positive for neuroendocrine markers. CD44 is positive in small cell carcinoma of prostate.

PSA and PSAP are either negative or only focally positive.

PROSTATIC CARCINOMA WITH SQUAMOUS CELL DIFFERENTIATION[42]:

This carcinoma is associated with poor prognosis.

Serum PSA levels may be normal. This is composed of glandular and squamous components[43,44] with infiltrating nests of malignant cells having abundant cytoplasm and intercellular bridges[45].

PSA and PSAP (prostatic specific acid phosphatase) is positive inmajority of cases, confirming it as prostatic origin.

UROTHELIAL CARCINOMA:

Primary urothelial carcinoma arises from prostatic urethra, periurethral glands and proximal prostatic ducts.

Secondary urothelial carcinoma involves the prostate by direct extension of an invasive urothelial carcinoma.

UROTHELIAL CARCINOMA INSITU

Prostatic ducts and acini are involved and filled with urothelial carcinomatous cells. They show marked nuclear and cytoplasmic atypia.

They arise denovo or extends from a bladder primary urothelial carcinoma.

Stromal invasion is seen with striking desmoplastic response.

CK7 staining is positive. Also positive for p63, thrombomodulin, GATA3, uroplakin and HMWK.

Prostatic markers like PSA, PSAP are negative.

NORMAL STRUCTURES MIMICKING PROSTATIC CARCINOMA

SEMINAL VESICLE/ EJACULATORY DUCTS

It is a paired tubular glands which secretes ejaculatory fluid that nourishes sperms and helps in sperm motility.

It is important to distinguish seminal vesicle/ ejaculatory duct epithelium from prostatic adenocarcinoma in needle biopsy specimens.

It is differentiated from prostatic adenocarcinoma by the presence of two types of cell layers which is confirmed by basal cell markers whereas it is absent in adenocarcinoma.

AMACR will be weak to negative in seminal vesicle unlike prostatic adenocarcinoma.

GANGLIA/ PARAGANGLIA/ PERIPHERAL NERVES

Ganglion cells are large cells having abundant pale eosinophilic cytoplasm with centrally placed nuclei and prominent nucleoli.

Paraganglion is composed of uniform population of cells having round centrally placed bland nuclei.

In contrast, prostatic adenocarcinoma have smaller, less uniform nuclei with large irregular nuclei and prominent nucleoli.

COWPER GLANDS/ BULBOURETHRAL GLANDS

They are pea sized glands located outside and lateral to the prostate gland which produces lubricant secretions. They are located within the skeletal muscle and has a lobular configuration.

It consists of large central ducts lined by bland cuboidal cells and are surrounded by small glands composed of cells having cytoplasmic mucin with basally located hyperchromatic nuclei.

PAS stain is positive in acini.

PSA marker is negative.

COLONIC GLANDS

Sometimes colonic mucosa is pushed into the core needle biopsy artifactually. It can mimic adenocarcinoma and is distinguished by the presence of mucin and goblet cells.

Also PSA and PSAP are negative and typically positive for CK20, CDX2.

SPREAD OF CARCINOMA

Invasion of tumor cells into periprostatic fat leads to perineural invasion.

It involves seminal vesicle along ejaculatory ducts, regional lymph nodes such as iliac nodes, common iliac nodes, paraaortic, hypogastric nodes.

It can metastasise to bone, liver, thorax, brain, GIT, retroperitoneum, kidney and adrenals.

TNM CLASSIFICATION OF PROSTATIC CARCINOMA

TX	-	Primary tumor cannot be assessed.
T0	-	No evidence of primary tumor
T1	-	Clinically inapparent tumor neither palpable nor visible by imaging
T1a	-	Tumor incidental histologic finding in 5% or less of tissue resected
T1b	-	Tumor incidental histologic finding in more than -5% of tissue resected
T1c	-	Tumor identified by needle biopsy (e.g., because of elevated PSA). Includes tumors found in both lobes by needle biopsy.
pT2	-	Tumor confined within the prostate
pT2a	-	Unilateral, one half of one side or less.
pT2b	-	Unilateral, involving more than one half of one side but not both sides.
pT2c	-	Bilateral (B/L) disease
pT3	-	Extraprostatic extension.
pT3a	-	Extraprostatic extension or microscopic invasion of bladder neck
pT3b	-	Seminal vesicle invasion

pT4 - Invasion of rectum, levator muscles, and/or pelvic wall'

Regional Lymph Nodes

NX - Regional nodes not sampled

N0 - No positive regional nodes

N1 - Metastases in regional node(s)

Distant Metastasis

M0 - No distant metastasis

M1 - Distant metastasis

M1a - Nonregional lymph node(s)

M1b - Bone(s)

M1c - Other site(s) with or without bone disease.'

GRADING OF PROSTATIC ADENOCARCINOMA

Donald F. Gleason in 1966, discovered a grading system for prostatic carcinoma based on architectural pattern of tumor.

Gleason score is obtained by combined gleason grade of gleason sum.

Gleason score ranges from 2 to 10.

Primary (predominant) pattern and secondary (2^{nd} predominant) patterns are identified and given a score from 1 to 5.

MODIFIED GLEASON GRADING[46]

Grading of prostate should be performed at low power in light microscopy[47,48].

PATTERN 1

Circumscribed nodule composed of closely packed, separate, uniform acini/ glands which are round to oval in shape.

PATTERN 2

Fairly circumscribed nodule composed of loosely arranged separate acini/ glands, not as uniform as gleason pattern 1.[49]

The edge of the tumor nodule may show minimal amount of infiltration.

PATTERN 3

Discrete glands which are smaller in size and infiltrates among the non neoplastic prostatic acini.

They are of varying size and shape[50]

PATTERN 4

They are composed of illdefined glands which have poorly formed glandular lumina and these glands are fused.

Cribriform pattern of glands are also seen.

Hypernephroid pattern (close resemblance to RCC) is also seen. These glomeruloid glands are characterized by dilated glands containing intraluminal cribriform structures with a single point of attachment which resembles glomeruli of the kidney.

PATTERN 5

No glandular differentiation is seen.

It is composed of solid sheets, cords, single cells.

Comedo carcinoma with central necrosis are noted.

PROGNOSTIC GRADE GROUPING[9]

‘Gleason score -> 2 to 6	--	group 1
3+4 = 7	--	group 2
4+3= 7	--	group 3.
8	--	group 4
9to 10	--	group 5’

AgNOR – Argyrophilic nucleolar organizer region staining[51]

Nucleolar organizer regions (NORs) occupies a specific portion in DNA called as rDNA which encodes rRNA (ribosomal RNA) for transcription.

This rRNA is responsible for the production of proteins in the cell, a step which is needed for cell proliferation[52,53].

Hence these NOR are related to the proliferative activity of the cell[54].

NOR is present in the short arm of acrocentric human chromosomes[3] 13, 14,15, 21, 22.

NORs are one of the portion in DNA present in the nucleoli that encodes for rRNA which forms the ribosomes, the "protein factories" of the cell.

NOR and its associated proteins have the capability to bind with silver. They are arygyrophilic .

It is visible under light microscopy as black intranuclear granules[55].

Nucleolin is a 92kd nucleolar protein[56] which is involved in rDNA transcription.

Phosphorylation of this protein leads to its activation and hence the transcriptionof rDNA.

This is performed by an enzyme which is a subunit of M phase kinase involved in bringing cells to mitosis.

Another protein called as Protein B23 is also involved in this process.

The ribosomal sequences of the chromatin is highly compact in resting cells whereas in case of increased proliferation, there is progressive dispersal due to increased synthesis of NOR[57,58].

Using AgNOR technique number, size and shape of silver dots are studied[59].

The amount of silver deposit reflects the NORs involved in protein synthesis and is related to the proliferative activity of the cell.

To score the amount of silver deposit, 100 cells are counted and a mean number of AgNOR dots per cell/ per nucleus is calculated.

The mean number of AgNOR dots in one nucleus is called as mAgNOR.

In non neoplastic / normal conditions, mAgNOR score ranges from 1- 5.

Percentage of cells that have >5 AgNORs per cell is called as AgNOR distribution score or AgNOR proliferation index (pAgNOR)[60] which indicates that these cells are in S phase of cell cycle having proliferative activity.

There is an inverse relationship between number of AgNORs and AgNOR size[61,62].

In malignant cells, number of AgNORs per nucleus will increase whereas the size of AgNOR dots is smaller and irregular[63,64].

In benign cells, the number of AgNOR dots are less and are larger and regular in size.

In case of cell maturation and cell differentiation in aging, the number and size of AgNOR dots decreases.

The size of the AgNOR dots are taken as smaller if dots are <3 microns and larger if >3 microns.

In malignancy, AgNORs moves from central to periphery inside the nucleus.

The shape of the AgNOR dots in malignancy tends to be predominantly small and have irregular, bizarre shape[65,66] whereas in benign, it is uniform, round and regular in shape[67].

AgNOR expression is seen in both proliferating and in resting cells[51].

AgNOR reflects only the process of protein synthesis not necessarily the cell proliferation.

It does not indicate the number of growing cells. It indicates the rapidity of the cell cycle and with tumor doubling time[68].

'GRADING OF AgNOR: (according to Ahsan et al)[69]

'The score of distribution for AgNOR dots are given as below:

0- More/ less uniform in size

1- Two different sizes of AgNOR dots

2- >two different sizes of AgNOR dots.

3- Including all grades and sizes.'

'The score of distribution for AgNOR dots dispersion are given as below:

0- Limited to nucleoli

1- Occasional dispersion outside nucleoli

2- Moderate dispersion outside nucleoli

3- Widely dispersion through the nucleolus.'

DISADVANTAGES:

Interobserver variations are common.

Counting procedures are done manually and hence it takes longer time which is a tedious process.

MATERIALS AND METHODS

Study Design

Prospective study

Study Period

From june 2016- may 2017

Study Place

Coimbatore Medical College& Hospital

Coimbatore.

Sample Size

A total number of 33 male patients.

From case records brief clinical data were collected, which includes age, sex, clinical diagnosis and surgical procedure.

The following inclusion and exclusion criteria were adopted.

Inclusion Criteria

Prostatic specimens like

1. Transurethral resection of prostate (TURP),

2. Needle biopsies

3. Prostatectomy specimens were included.

Exclusion criteria

Poorly preserved prostatic specimens and inadequate biopsies.

Methods

Of the total cases received in the department of Pathology in our hospital 33 cases were taken into the study according to inclusion criteria during the study period.

All those 33 prostate specimens were selected and then fixed with 10% formalin, embedded in paraffin and stained with haematoxylin and eosin.

HAEMATOXYLIN AND EOSIN STAINING METHOD

REAGENTS USED

1.Haematoxylin solution-‘ Erhlich’s haematoxylin’

2. Eosin Y 1% solution

3. Acid alcohol 1% solution.

PROCEDURE

1. ‘Deparaffinize sections
2. Immerse the sections in xylene for 30 minutes.
3. Place the sections in isopropyl alcohol in 15 minutes
4. Wash in running tap water
5. Stain in ‘Erhlich’s haematoxylin’for 10 to 15 minutes.
6. Differentiation is done with 1% acid alcohol two to three dips.
7. Blueing is carried out for 10 minutes.
8. Counterstain with eosin 1% solution 2 to 3 dips.

9.Running tap water wash.

10.Air dry the sections

11.Mount with DPX'

After haematoxylin and eosin staining, all slides were reviewed by pathologist and categorized as the following:

Benign prostatic adenomyomatous hyperplasia

Prostatic intraepithelial neoplasia

Prostatic adenocarcinoma

AGNOR- Argyrophilic nucleolar organizer region staining technique

Here, single step AgNOR staining technique was used by cutting paraffin sections of 3microns thickness to demonstrate AgNOR.

Silver nitrate method for AgNOR demonstration:

Sections

Formalin- fixed, 2-3 um paraffin sections.

Solutions

'50%silver nitrate solution

Silver nitrate- 50g

Distilled water- 100ml

Gelatin solution

Gelatin- 2g

Formic acid- 1ml

Distilled water- 100ml’

WORKING SOLUTION

Silver nitrate solution 2 parts by volume

Gelatin solution 1 part by volume

Mix the above proportion freshly before use.

STAINING METHOD

1. Dewax sections in xylene, then hydrating through ethanol and distilled water.
2. Rinsing sections in distilled water.
3. Incubate the slides in freshly prepared working solution for 30 to 45 minutes at room temperature.
4. Wash in distilled water for 1 minute.
5. Dehydrate, clear and mount in non-aqueous mounting medium.

INTERPRETATION

AgNOR sites- Intranuclear black dots.

Background- yellow.

OBSERVATION AND RESULTS

INCIDENCE OF VARIOUS PROSTATIC LESIONS

Totally 33 specimens received in the department of pathology Coimbatore medical college and hospital during the period june 2016-may 2017 were taken for the present study.

This prospective study included various prostatic biopsies from 33 patients which was diagnosed histologically during the period june 2016-may 2017.

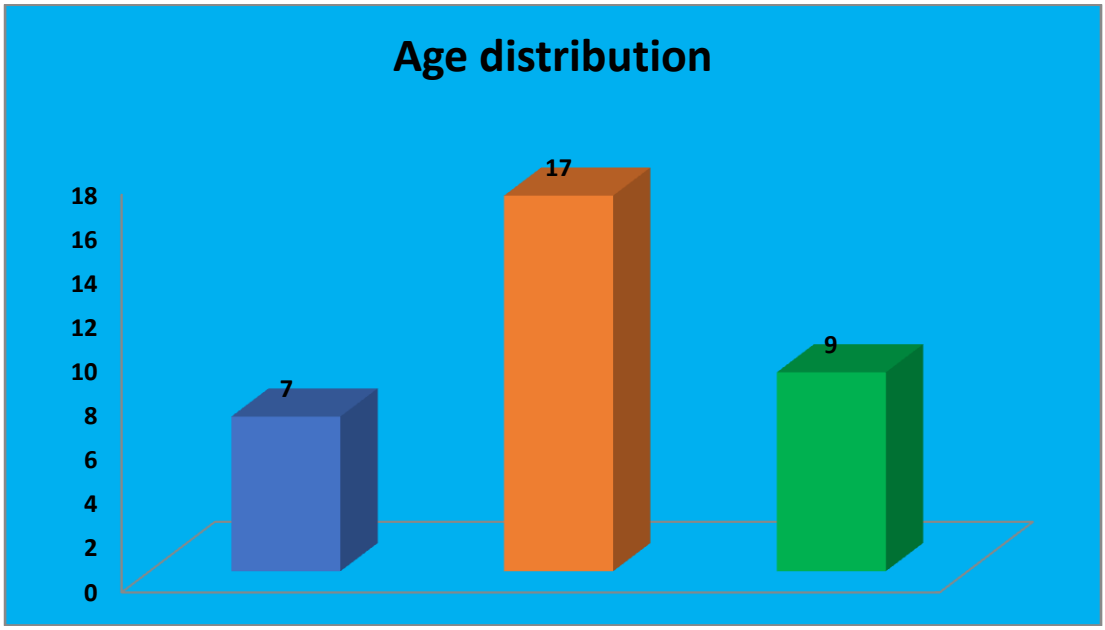
Table 1: Mean age of the study

	Number of cases	**Minimum**	**Maximum**	**Mean**	**Standard Deviation(SD)**
AGE	33	45	85	66.52	8.98

The minimum age limit was found to be 45 years in patients with prostatic disease.

Table 2: Age wise distribution of prostatic lesions

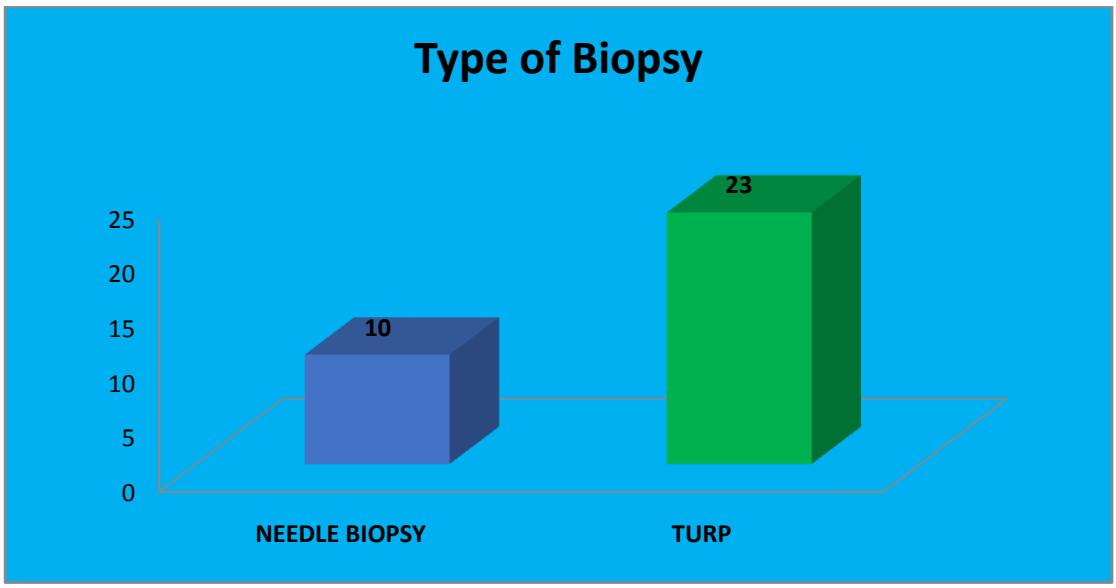
Age distribution	Number of cases	Percent (%)
50-60	7	21.2
61-70	17	51.5
71-80	9	27.3
Total	33	100.0



Incidence of prostatic lesions are more common in the age group ranging from 61-70 years of age and lowest in the age group of 50-60 years.

Table 3:Type of Biopsy in prostatic lesions

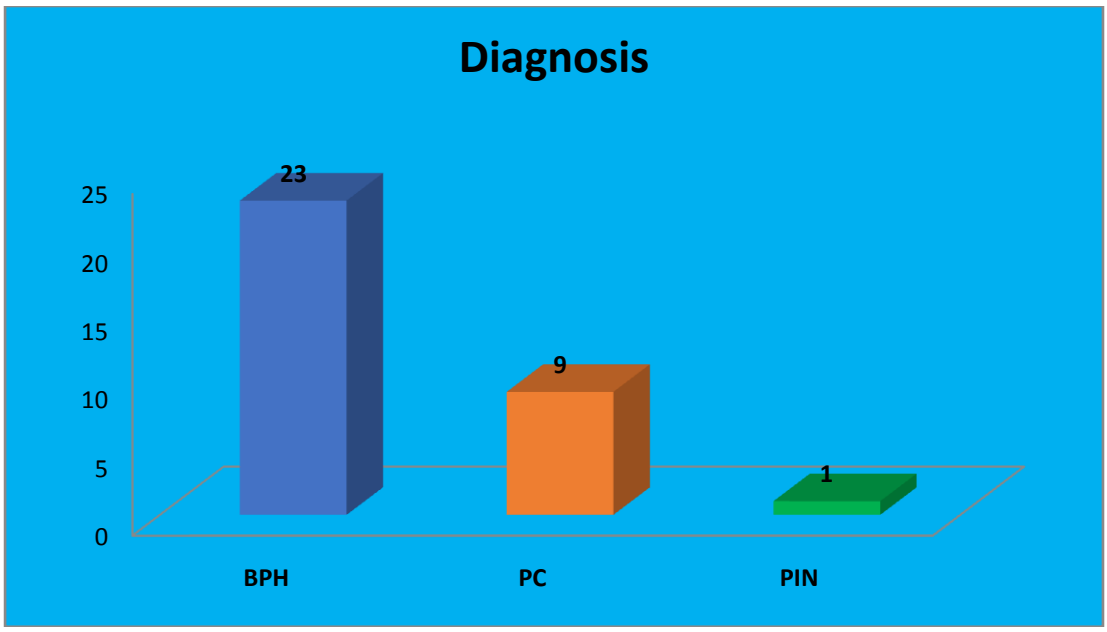
Type of Biopsy	Number of cases	Percent (%)
NEEDLEBIOPSY	10	30.3
TURP	23	69.7
Total	33	100.0



Among 33 specimens which was received in department of pathology, Coimbatore medical college and hospital, majority of the specimens were TURP followed by needle biopsies.

Table 4:Diagnosis of various prostatic lesions:

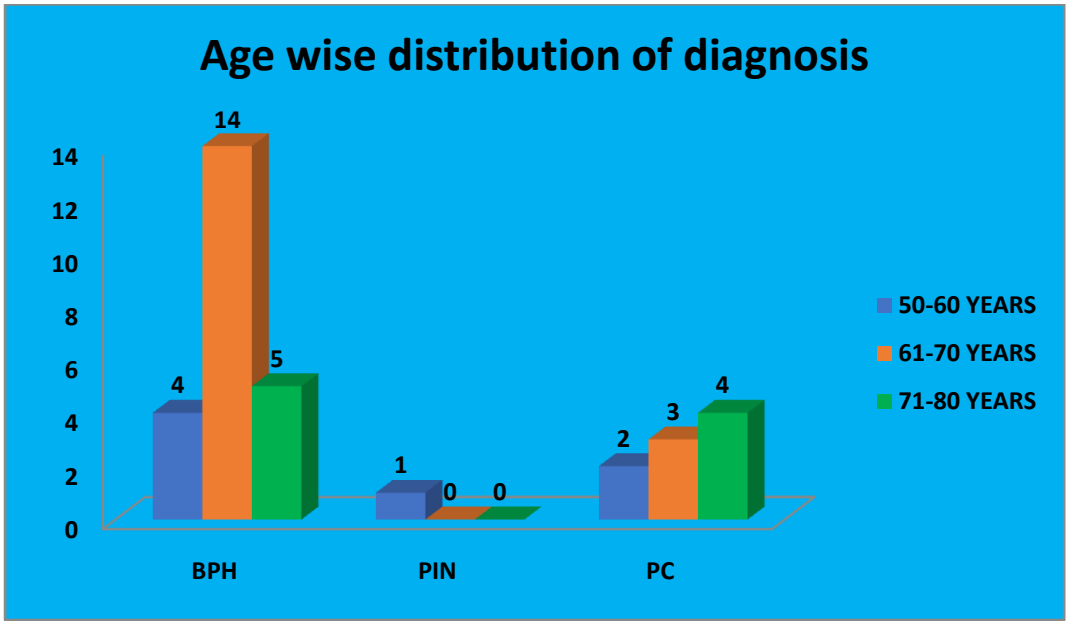
Diagnosis	Number of cases	Percent (%)
BPH	23	69.7
PC	9	27.3
PIN	1	3.0
Total	33	100.0



The incidence of Benign prostatic Hyperplasia was highest in the study with 23 cases contributing to 69.7 %, followed by 9 cases of prostatic carcinoma comprising of 27.3 % of cases and one case ofProstatic intraepithelial neoplasia constituting 3%.

Table 5: Age wise distribution of various prostatic lesions:

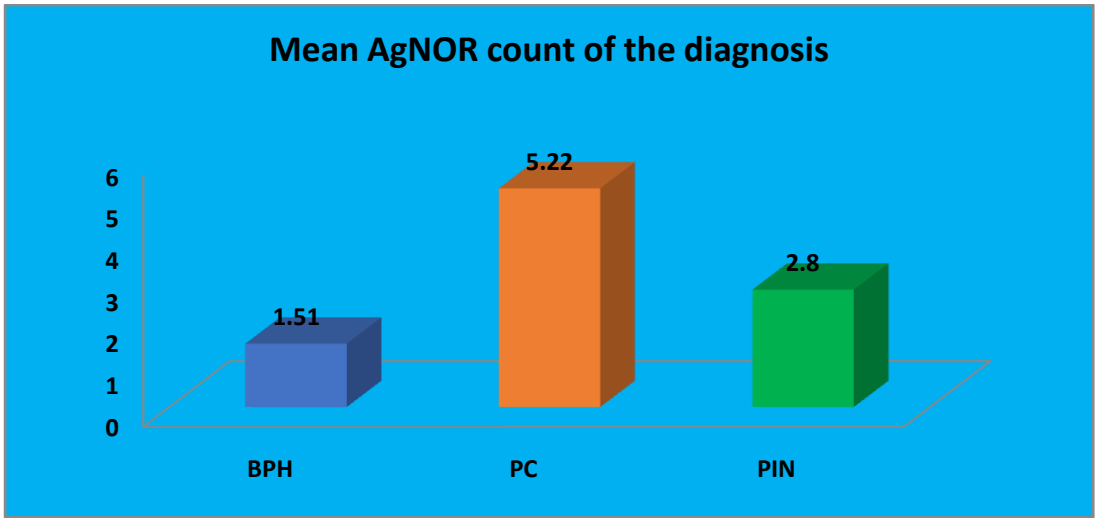
Age distribution	BPH	PIN	PC
50-60	4(57.1%)	1(14.3%)	2(28.6%)
61-70	14(82.4%)	0(0.0%)	3(17.6%)
71-80	5(55.6%)	0(0.0%)	4(44.4%)



BPH was the most common lesion in all age groups. However the incidence of prostatic carcinoma increased in the age group 71-80 years constituting 44.4% and BPH constitutes 55.6% in this age group.

Table 6: Mean AgNOR COUNTof various prostatic lesions:

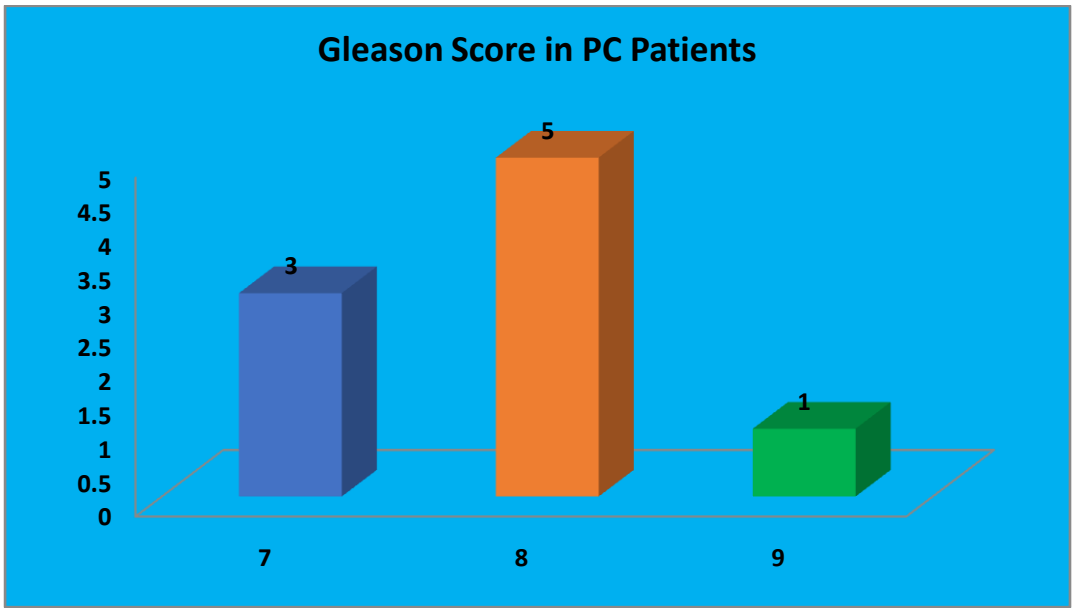
	Number of cases	MeanAgNOR count	Standard Deviation(SD)
BPH	23	1.51	.21
PC	9	5.22	.14
PIN	1	2.80	-



In the present study, mean AgNOR count in Benign prostatic hyperplasia is low and found to be 1.5 whereas in prostatic adenocarcinoma it is highest of about 5.22 .

Table 7: Gleason Score in PC Patients

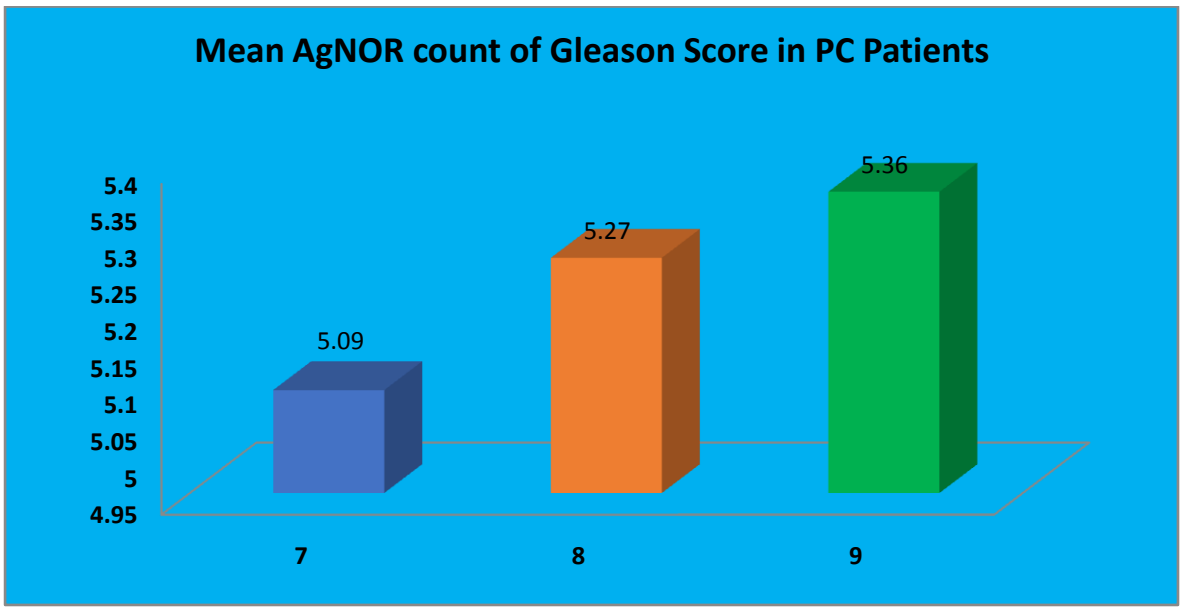
Diagnosis	GLEASON SCORE		
	7	8	9
PC	3(33.3%)	5(55.6%)	1(11.1%)



In the present study, majority of the patients of prostatic carcinoma falls in the category of gleason grade 8 and one case in gleason grade 9.

Table 8: Mean AgNOR COUNTof Gleason Score in PC Patients

Gleason Score	Number of cases	Mean AgNOR count	Standard Deviation(SD)
7	3	5.09	.18
8	5	5.27	.05
9	1	5.36	-



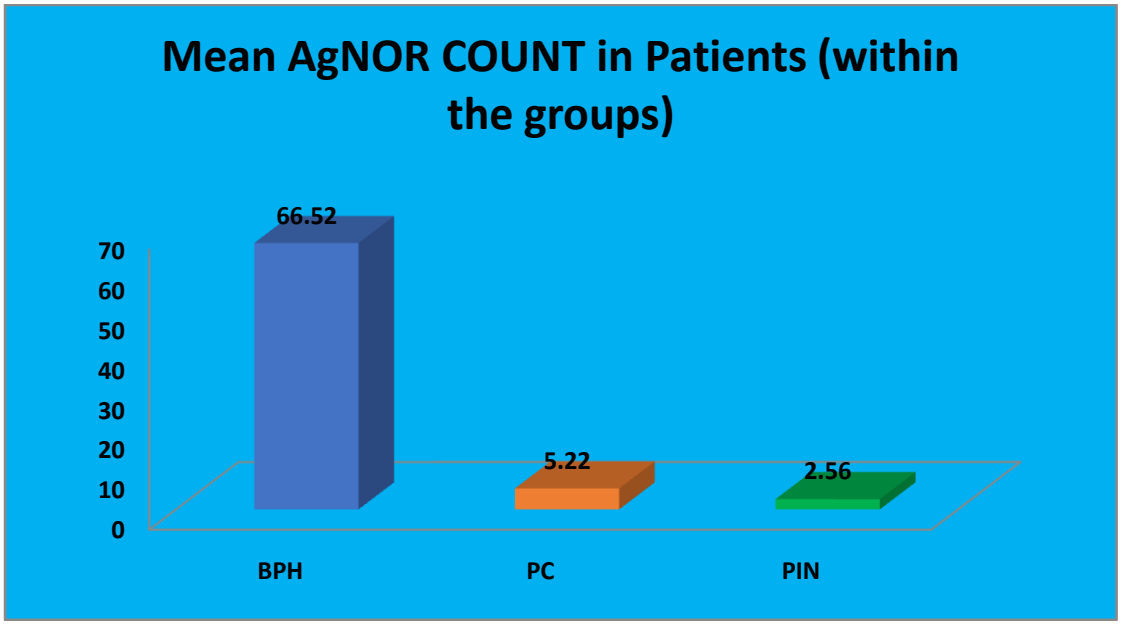
Mean AgNOR count is found to be highest in gleason score 9 compared tolowest gleason score in the present study.

Table 9: Mean AgNOR COUNTin various prostatic lesions

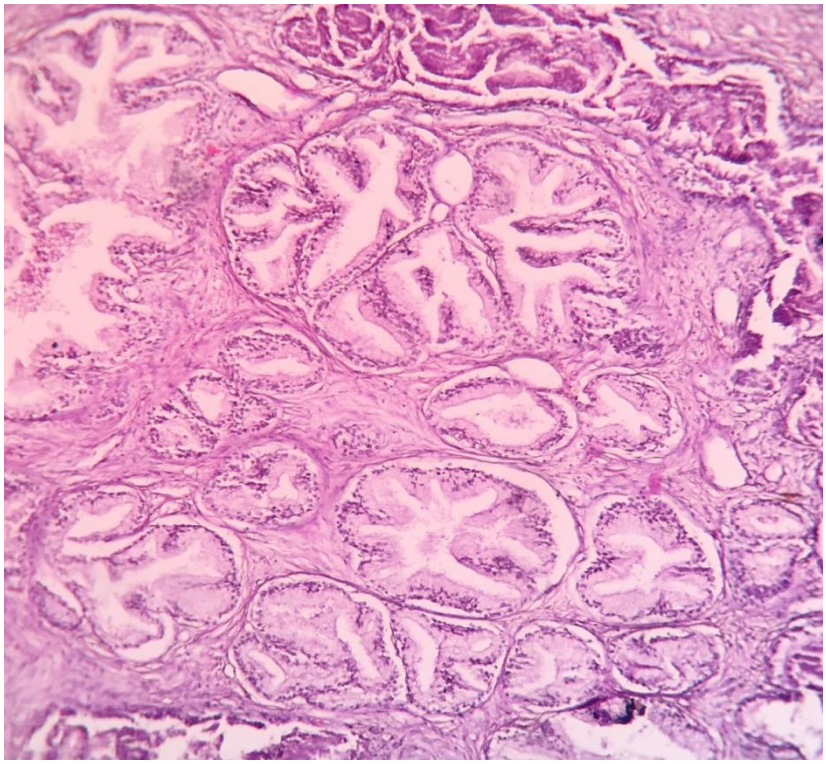
(within the groups) using ANOVA

	No of cases	Mean AgNOR score	Standard Deviation(SD)	95% Confidence Interval for Mean		P value
				Lower Bound	Upper Bound	
BPH	23	66.52	8.98	1.42	1.61	.000*
PIN	1	5.22	.14	5.11	5.33	
PC	9	2.56	1.67	1.97	3.16	

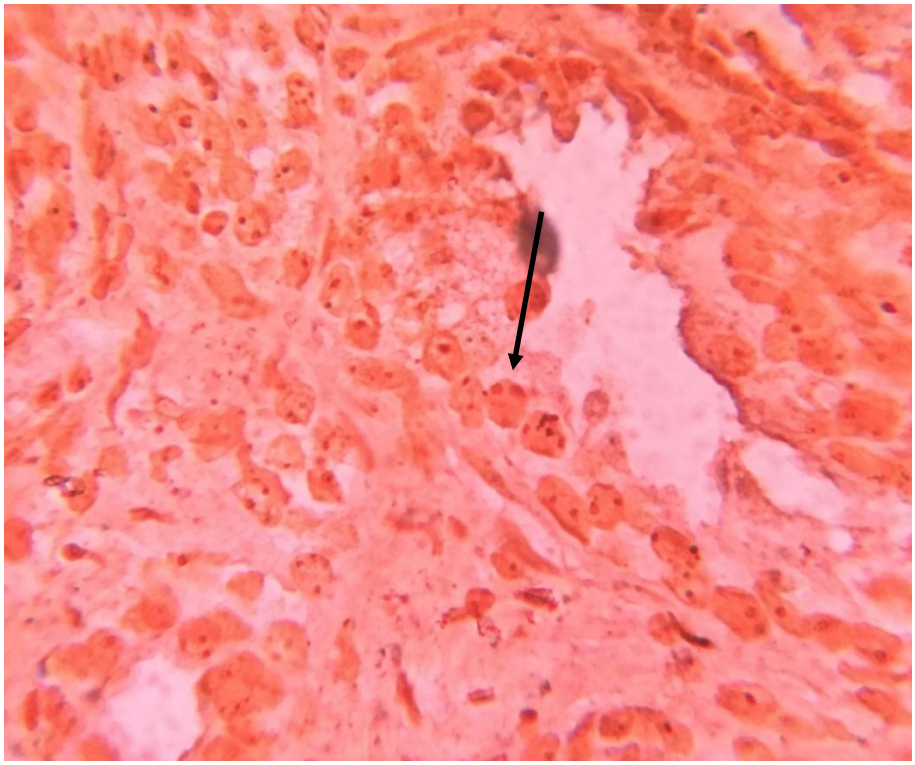
*STATISTICALLY SIGNIFICANT (P<0.05)



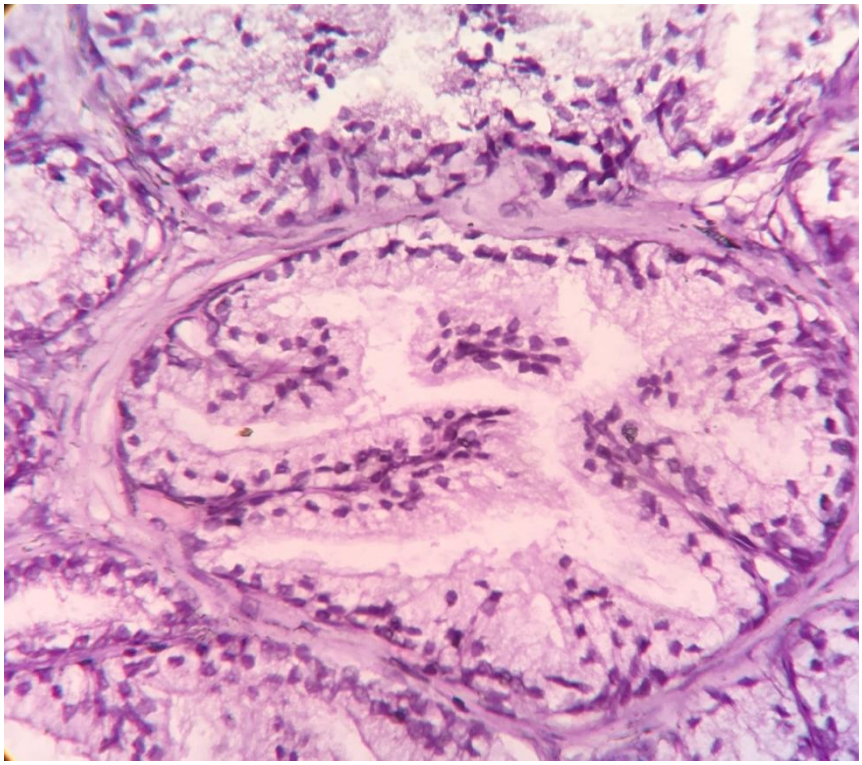
From the statistical analysis of the present study, AgNOR staining is found to be both sensitive and specific marker to distinguish benign and malignant prostatic lesions.



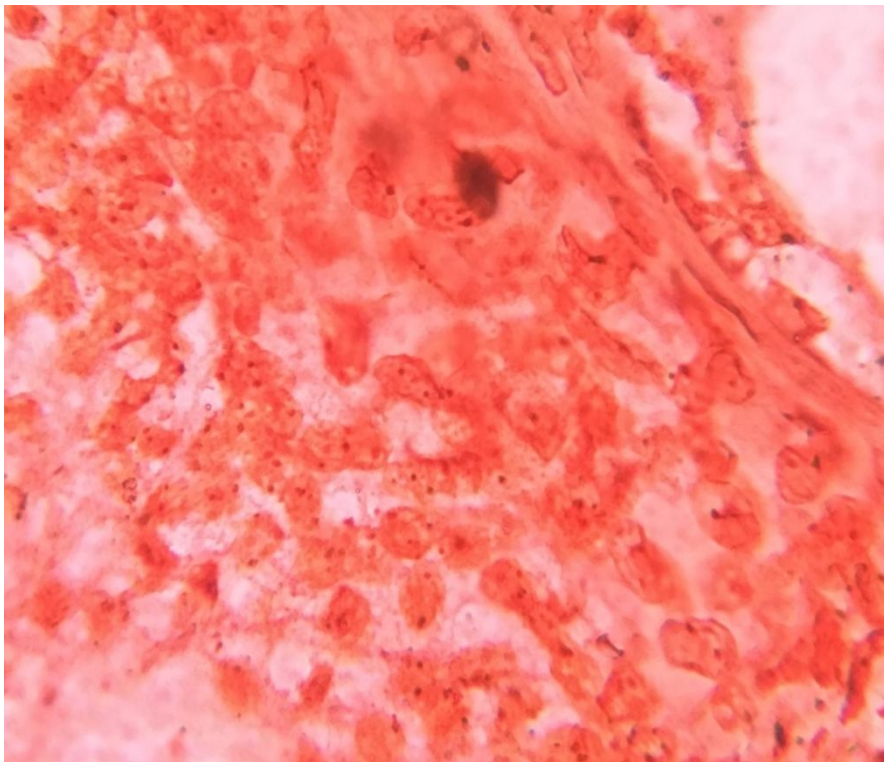
Benign Prostatic Hyperplasia- H&E Stain 10x magnification.



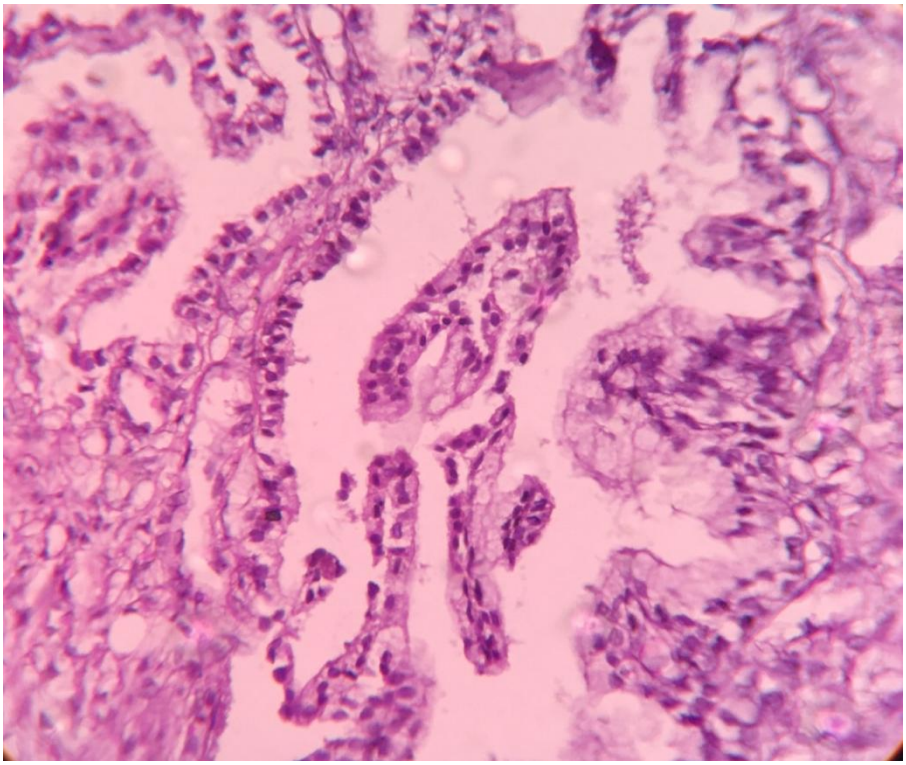
Benign Prostatic Hyperplasia- AgNOR dots are regular,homogenously stained inside the nucleus.



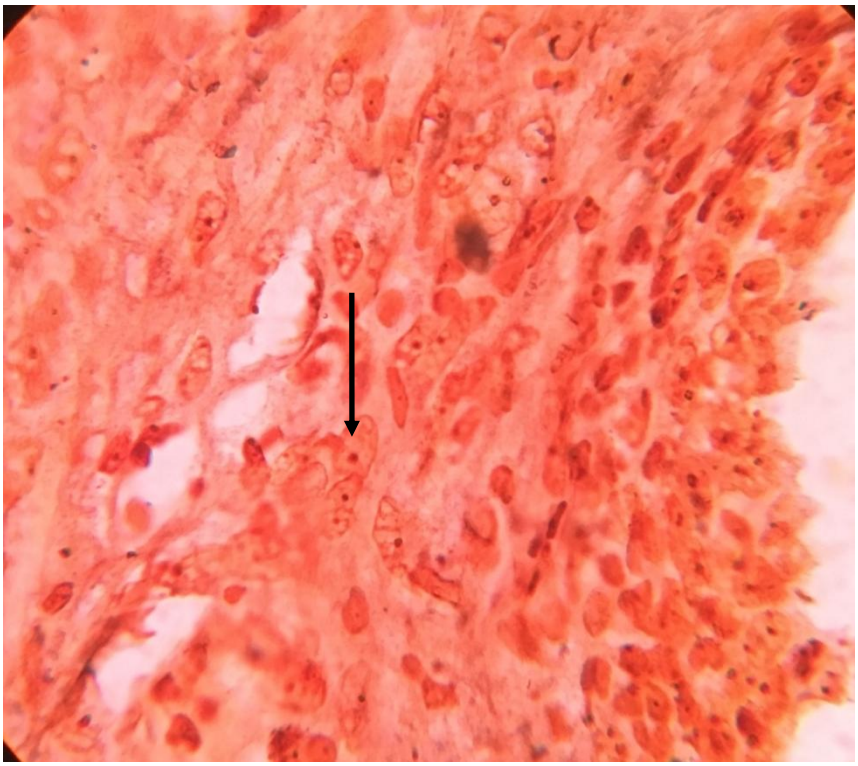
Benign Prostatic Hyperplasia - H&E stain 40x.



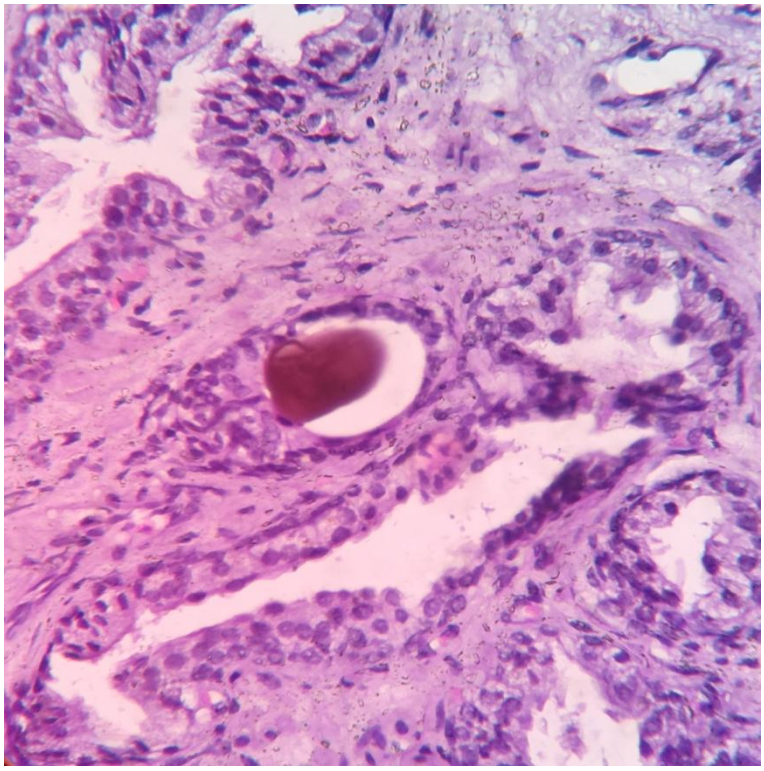
AgNOR dots in Benign Prostatic Hyperplasia.



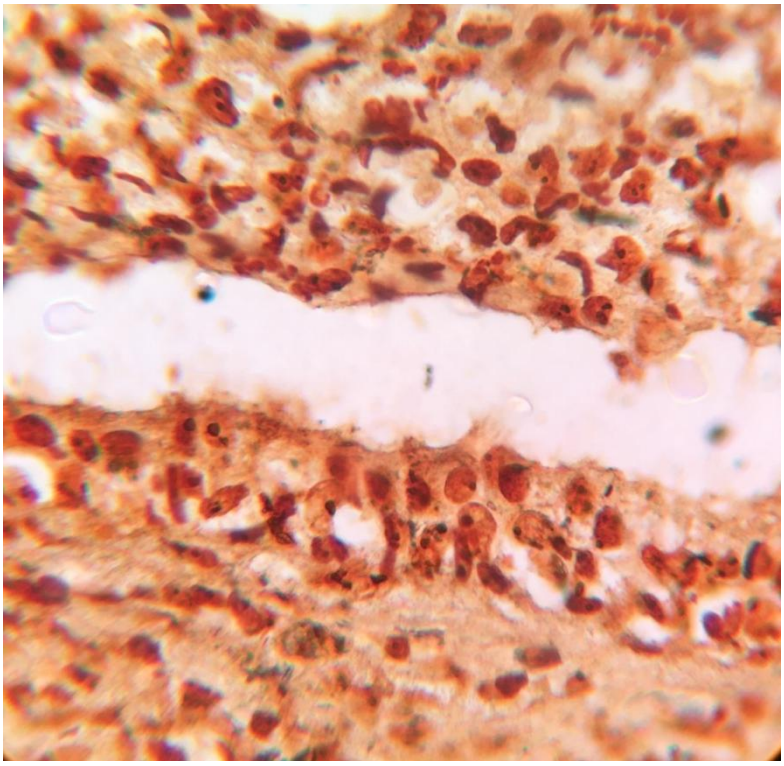
Benign Prostatic Hyperplasia –H&E stain.40 x.



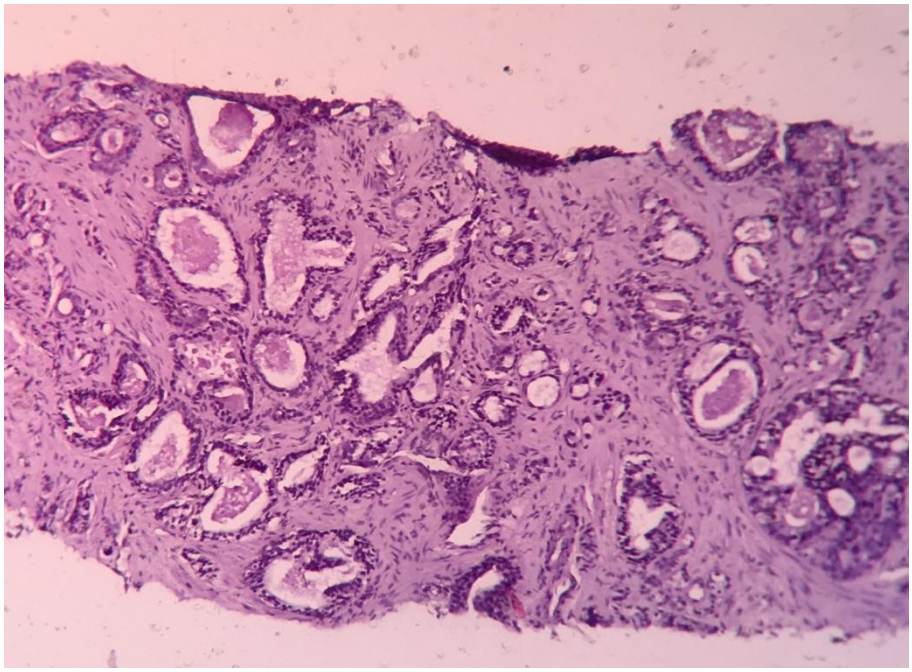
Benign Prostatic Hyperplasia- Large, Regular, round AgNOR dots.



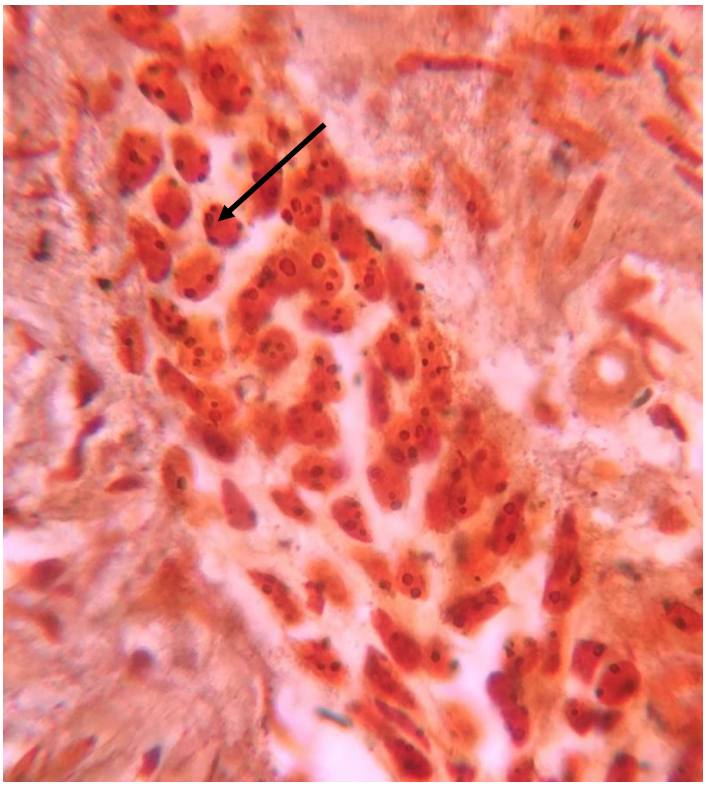
Benign Prostatic Hyperplasia H&E stain. 40X magnification.



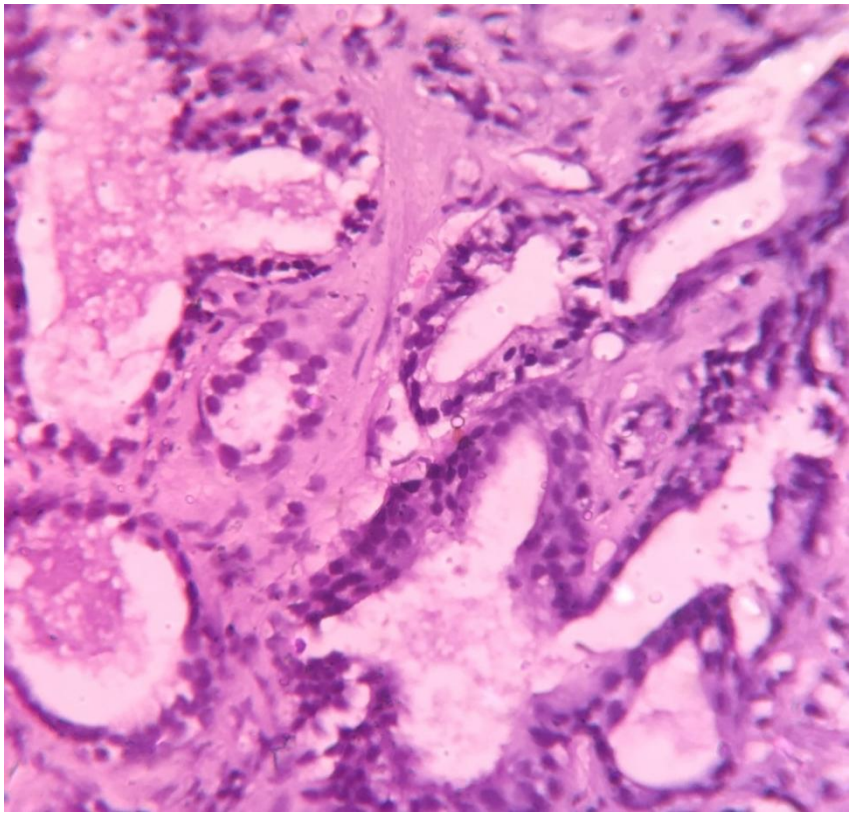
AgNOR dots in Benign Prostatic Hyperplasia- Large, Regular, round and homogenously stained.



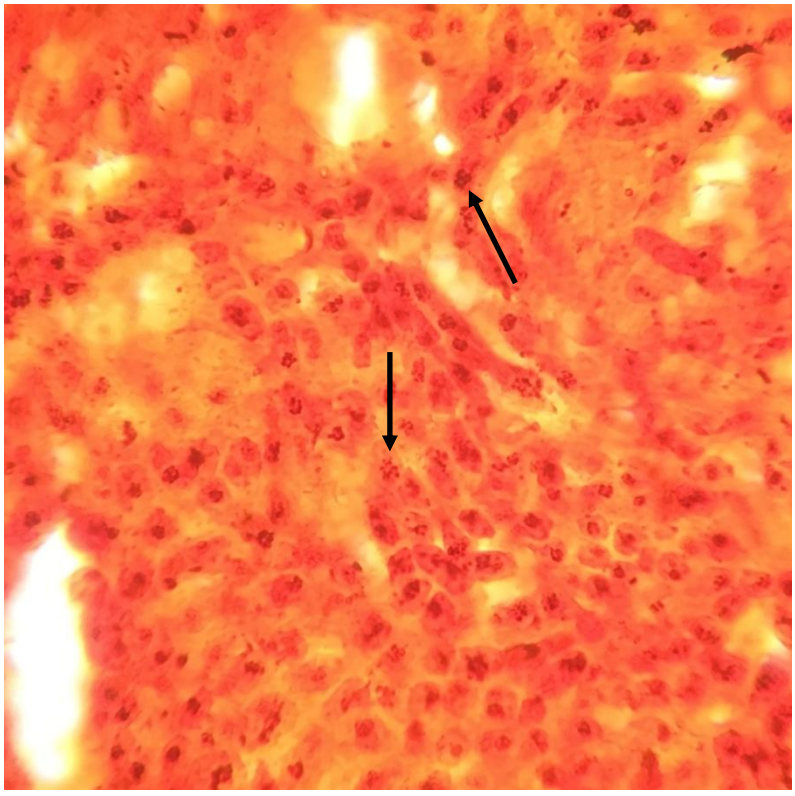
PROSTATIC ADENOCARCINOMA (H&E STAIN) 10X



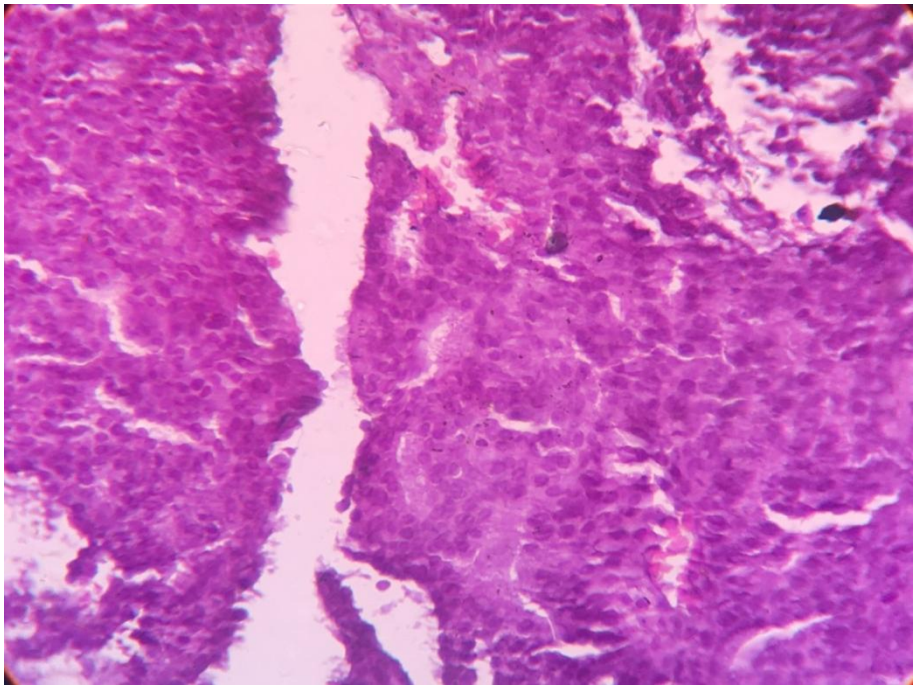
AgNOR dots (in adenocarcinoma) Gleason score 7 – irregular, variable size and shape.Arrow – dots stuck to the periphery of the nuclear membrane.



Prostatic adenocarcinoma- H&E stain.40x magnification.

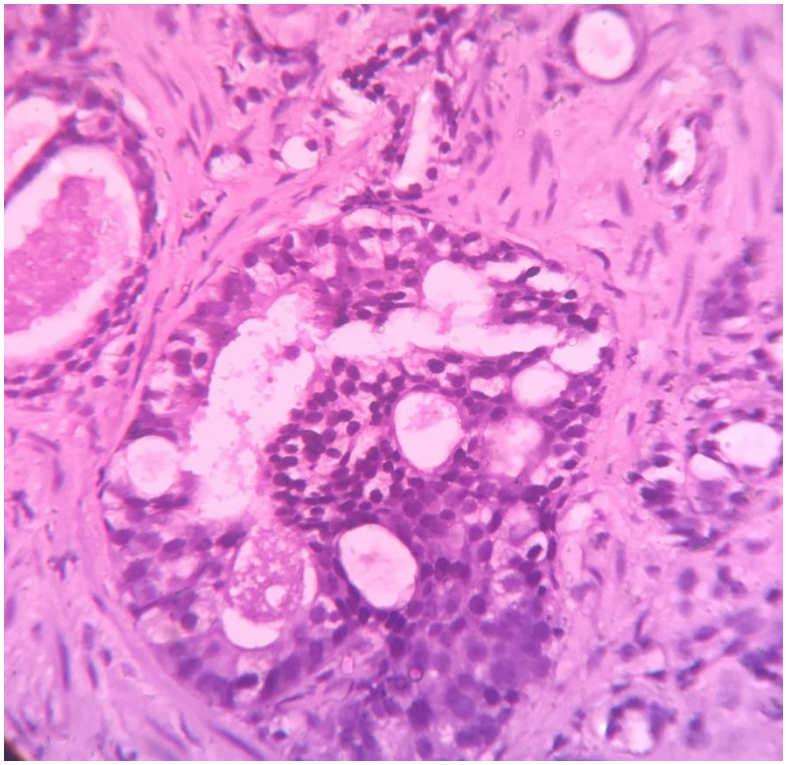


Prostatic adenocarcinoma : Gleason Score 9- Individual AgNOR dots are smller in size ,more in number and appear fused and bizarre.

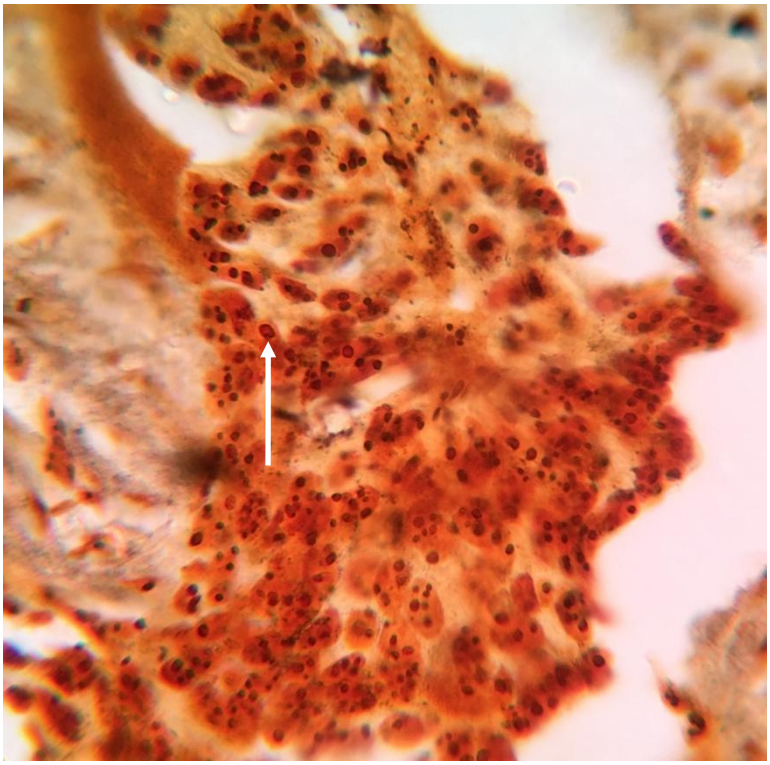


Prostatic Adenocarcinoma –H & E stain (40x)

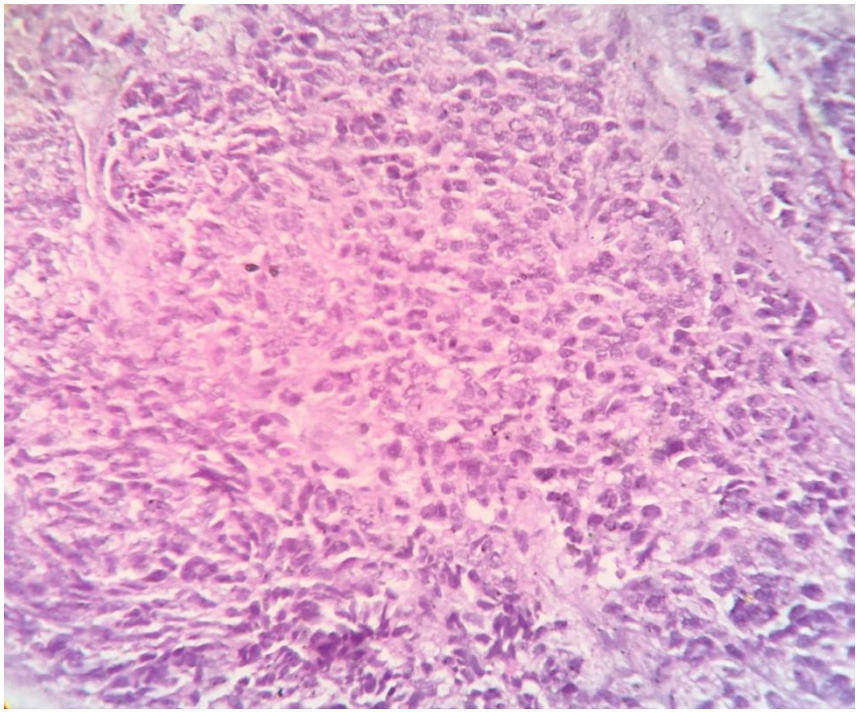
Prostatic Adenocarcinoma :Gleason score 8- AgNOR in prostatic adenocarcinoma- Increased number of dots of varying size with irregular shape in the nucleus.



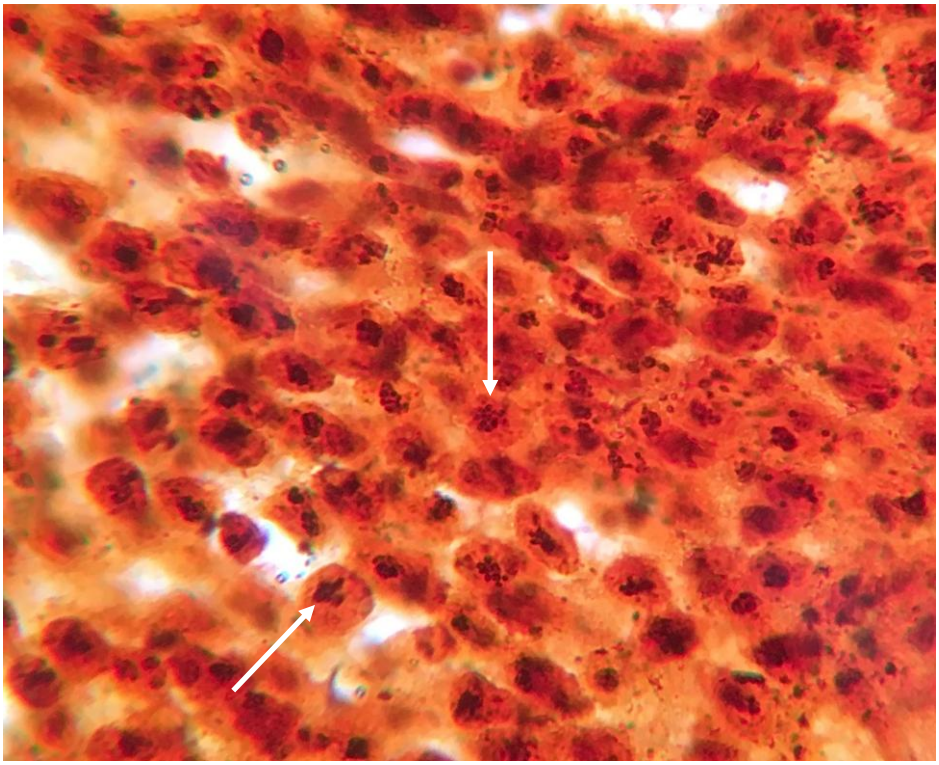
Prostatic Adenocarcinoma –H & E stain (40x)



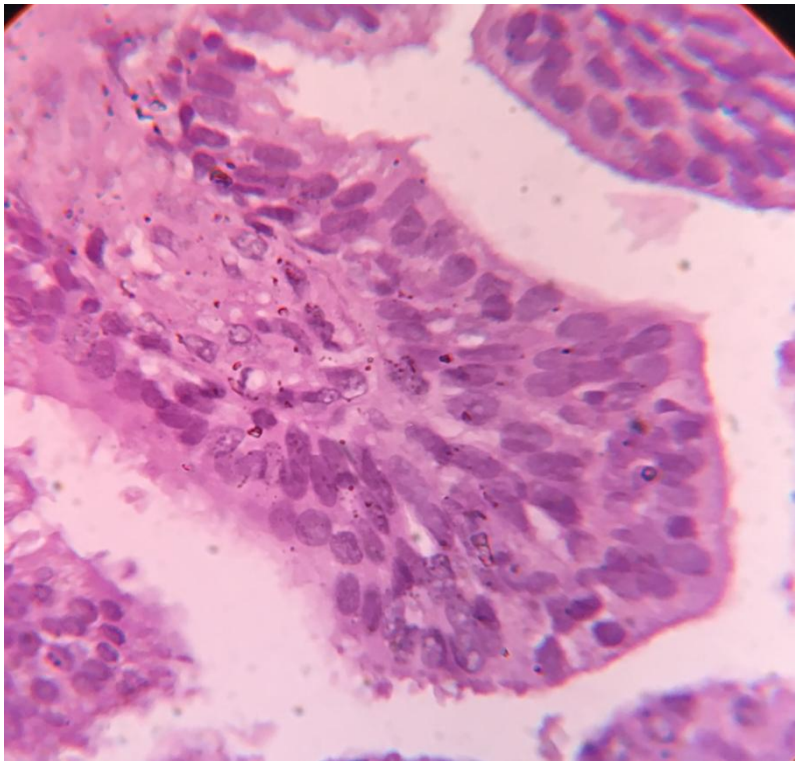
Prostatic Adenocarcinoma: Gleason Score 8- AgNOR dots are increased in number with irregular contour.



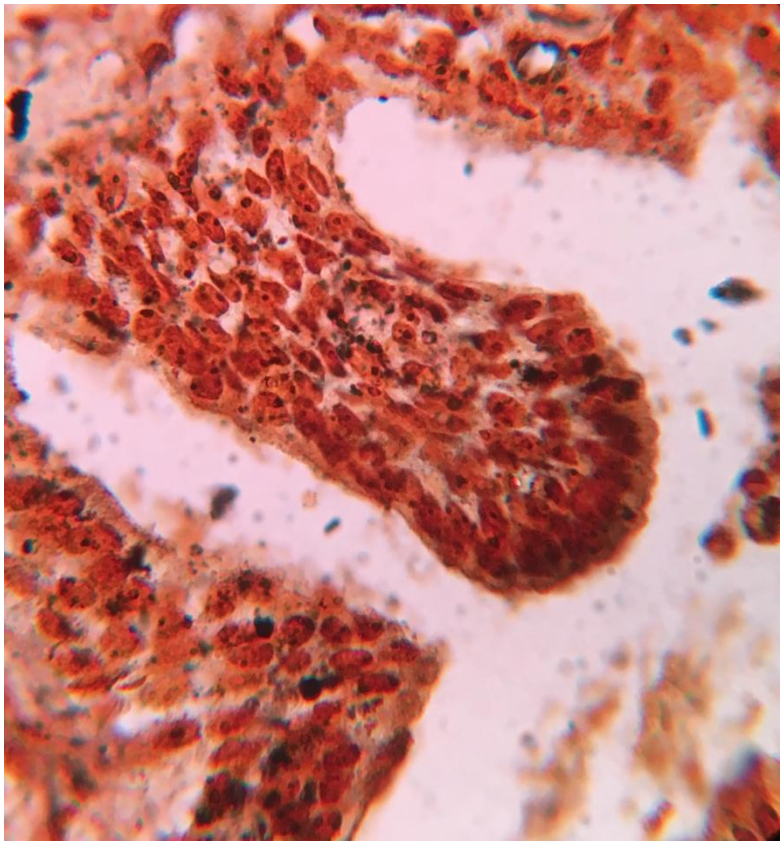
Prostatic Adenocarcinoma –H & E stain (40x)



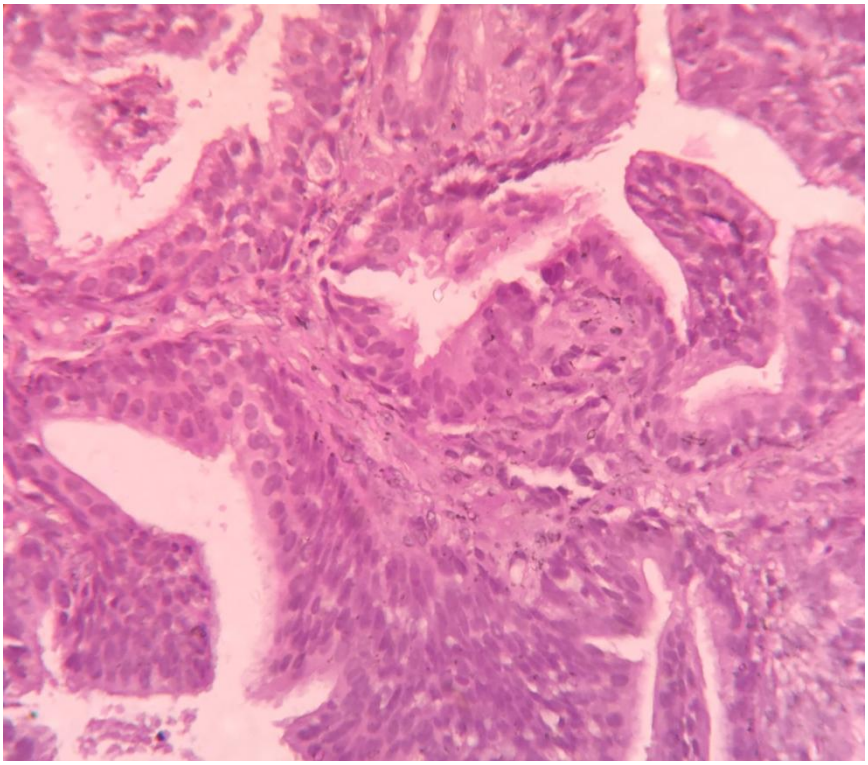
Prostatic Adenocarcinoma : Gleason Score 9 - Individual AgNOR dots are obscured and appear fused. Few giant and bizarre dots are seen.



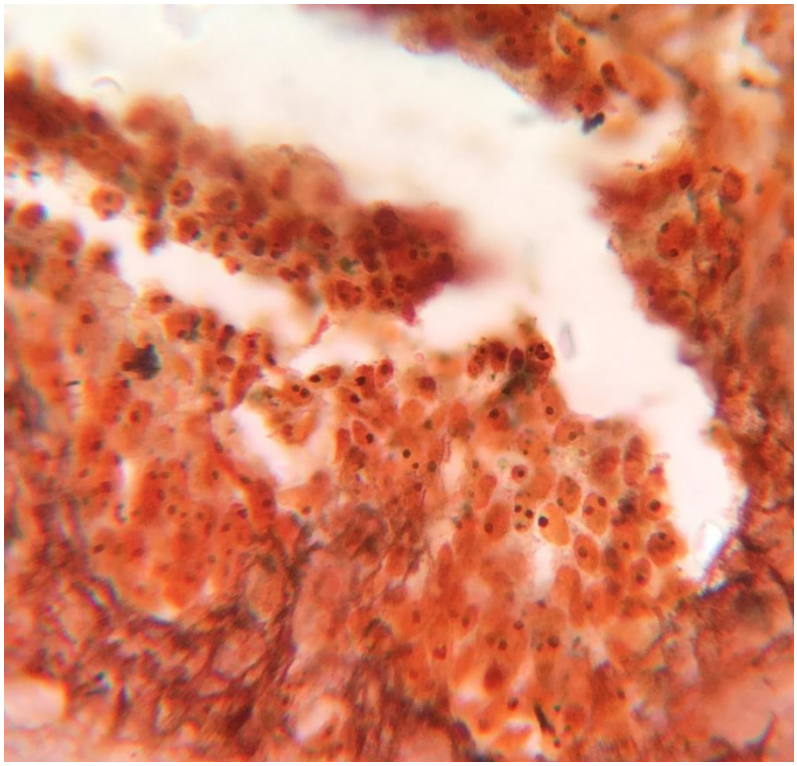
Prostatic Intraepithelial neoplasia- H & E(40x)



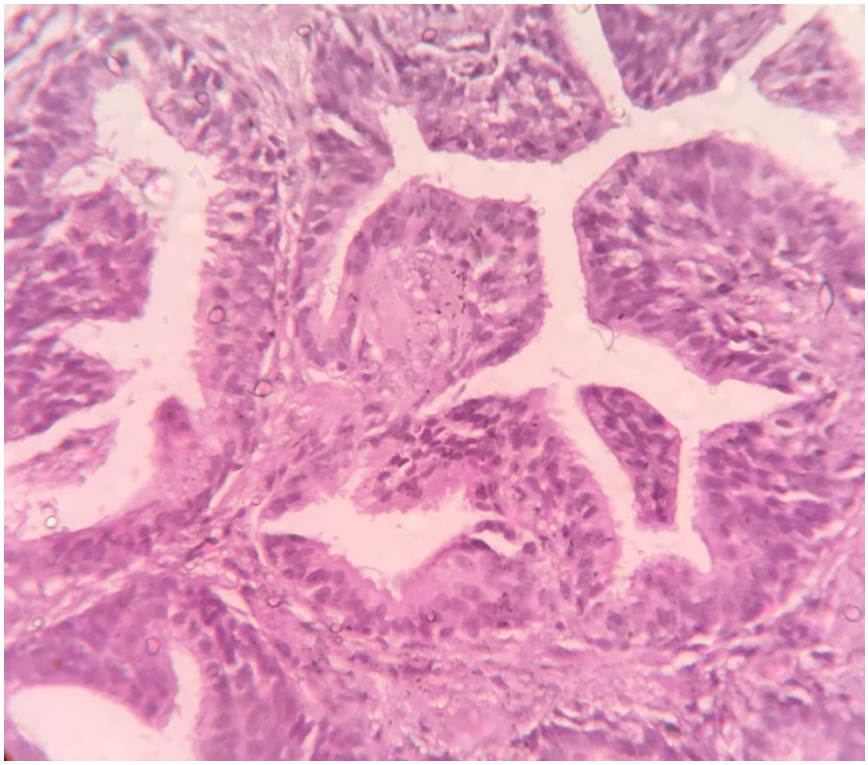
AgNOR dots in Prostatic Intraepithelial Neoplasia (PIN)



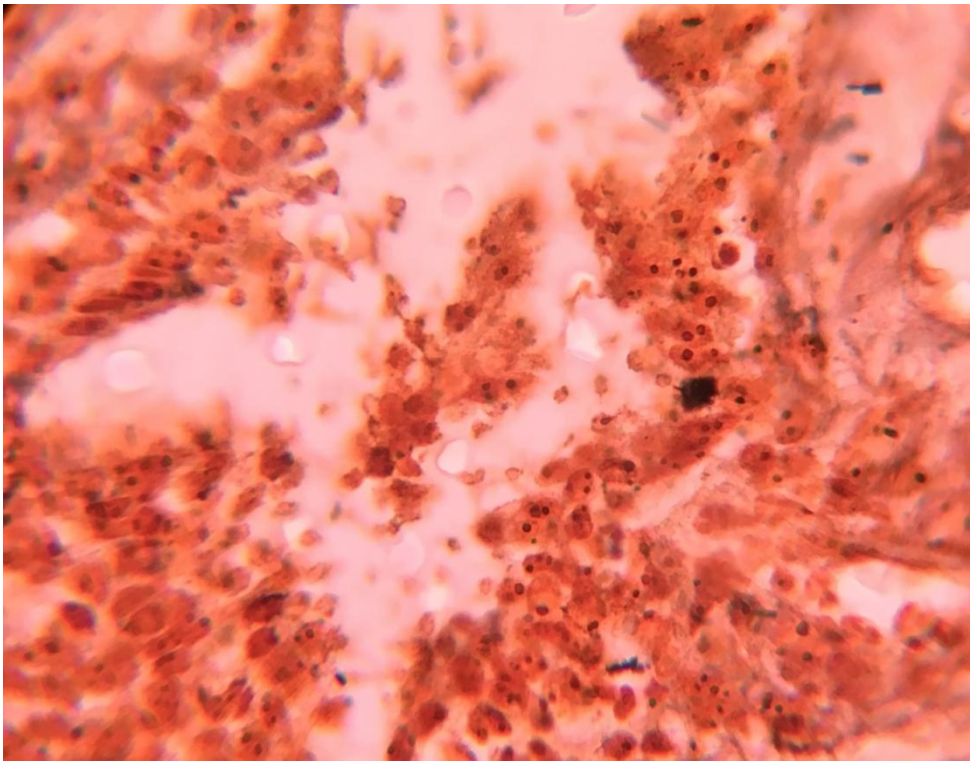
Prostatic Intraepithelial Neoplasia H&E stain 40x.



Prostatic Intraepithelial Neoplasia- Increased number of AgNOR dots.



Prostatic Intraepithelial Neoplasia. H&E stain 10x



mAgNOR count of 2.8 in Prostatic Intraepithelial Neoplasia.

DISCUSSION

Prostatic carcinoma is one of the most common cancer among men and second leading cause of death among the various cancers[70].

Early diagnosis and prompt treatment of prostate cancer is essential to benefit the patients.

Histologic grade is the important parameter to assess the prognosis of prostate cancer patients. But the tumor proliferative activity is difficult to assess[8].

The present study was carried out to evaluate the increased cell ploidy and increased transcriptional activity by silver staining for the NOR.

Demonstration of NOR by one stage argyrophilic method is useful to assess the prognosis of the patient[59].

The phase of the transcriptional activity of the cell is denoted by the size, number of AgNOR dots.

It significantly differs from benign prostatic lesions and helps in differentiating among various prostatic lesions.

It stains as black dots inside the nucleus of the cell[60].

AgNOR method

In the present study, sections were cut at 3 microns thickness and fixed in 10%formalin.

AgNOR dots are best visualized in thin sections.

AgNOR Counting

In counting AgNOR, the black dots are counted under light microscopy[59]and oil immersion.

AgNOR counts can also be studied by image cytometry. It has higher degree of precision with minimal interobserver variations.

AgNOR is usually indiscernible in normal cells because of tight packing of nucleoli inside the nucleus.

In malignancy due to the increased proliferation of neoplastic cells, nucleolar disaggregation occurs and leads to dispersion of AgNOR[71].

The present study highlights the importance of AgNOR in differentiating benign, premalignant and malignant lesions of the prostate

This method highlights the rapidity of cell proliferation and its prognostic significance.

Counting is done at higher magnification (100x oil immersion).

AgNOR are seen as black dots inside the nucleus[72].

In the present study, the AgNOR dots are large, regular in benign prostatic hyperplasia whereas the dots are irregular in shape and small in size with giant fused bizarre clusters and appear blotchy in prostatic carcinoma.

Stella Mamaeva et al study revealed that adenomatous samples displayed few AgNOR s (mean 13 dots/ cell) than did the carcinomas (mean 24 dots/ cell).

In the present study, the. Number of dots in benign lesions is 2-3/cell whereas in malignancy, it is 6-8 dots /cell and in prostatic intraepithelial hyperplasia, it is 3-4 dots/cell.

In the present study, most of the dots were homogenous and had regular contours in benign lesions, whereas in prostatic carcinoma, it is asymmetric, heterogenous and had irregular contours[51].

They are smaller, variably scattered and are aggregated.

Rajeshwari et al[8] done a prospective study on various prostatic lesions and found that the mean AgNOR count in benign lesions is 1.6+/-0.2 and AgNOR tend to be larger, regular and homogenously stained.

The mean AgNOR count in malignant lesions was between 4.7 +/- 0.1 to 5.7 +/-0.9 and the dot appears to be large, irregular in size and shape with giant and bizarre clusters.

In the present study, it is also noted that the size of the AgNOR dots decreases with malignancy when compared to benign prostatic lesions.

In the present study, mean AgNOR count in benign prostatic hyperplasia is1.51 and in prostatic adenocarcinoma it is 5.22.

AgNOR counts have prognostic significance .

Higher AgNOR (intranuclear black dots) counts have poor prognosis when compared to lower AgNOR values.

M.Ghazizadeh et al[59] demonstrated silver staining of nucleolar organizer regions in prostatic lesions and found that the mean AgNOR count significantly increased with increasing gleason's grade ($p<0.01$) and clinical stage ($p<0.05$) of the tumors.

AgNOR counting may contribute to the conventional diagnosticand prognostic indices of cancer of the prostate.

K.Subathra and N.Sangeetha et al analysed in their study that mean AgNOR counts were higher in malignant lesions when compared with benign lesions.

Takemasa ohki et al demonstated that tumors having greater number of AgNORs have poorer prognosis.

Norio Kawase et al studied nucleolar organizer regions in 79 prostatic cancers and revealed that AgNOR counts were diagnostically and prognostically more valuable.

The survival of patients with higher AgNOR counts (>4.3) wassignificantly poorer than survival of those with lower AgNOR counts (<4.3).

Sakr et al[6] using 20 specimens showed a significant correlation between AgNOR expression and gleason score and concluded that AgNOR could be a marker of tumor differentiation.

In the present study, it was observed that mean AgNOR increases as the gleason grade increases. mAgNOR count for gleason score 7 ranges from 4.88 to 5.20 and it is 5.36 in gleason score 9.

Sadia Hameed, Anushree C.N et al[55] studied that among the prostatic specimens, TURP chips was the most common specimens accounting for 83.2 % of the cases.

In the present study, TURP specimens was the most common and constitutes about 69.7% .

Carter and colleagues showed that 50% of men between 70 and 80 years of age showed histological evidence of malignancy.

In the present study also, the incidence of prostatic carcinoma is highest in the age group of 71- 80 years .

Mittal[72] and Anushree et al demonstrated that the age range of the patients was between 61 to 70 years in benign lesions and between 71 to 80 years in malignant lesions.

In the present study also, benign prostatic hyperplasia is common in the age group of 61-70 years and prostatic adenocarcinoma is common in the age group of 71-80 years which is in accordance with the above study.

In Mittal and Anushree et al[72] study, benign lesions were most common accounting for 90.4 % followed by prostatic adenocarcinoma accounting for 9.6% of cases and in our study also, benign prostatic hyperplasia constitutes about 69.7 % and prostatic carcinoma constitutes for about 27.3% which is almost similar to the above study.

The present study was completed successfully by giving importance to the quality of reagents, cleanliness and purity of the

materials used during the procedure, correct temperature, accurate duration of the staining.

Water purification was done to avoid the background staining and nonspecific granular deposits in tissue sections.

AgNOR scores help to differentiate between benign prostatic hyperplasia, prostatic intraepithelial neoplasia and prostatic adenocarcinoma and AgNOR score correlate with the Gleason score and thus helpful in assessing the severity of the tumor and thereby prognosis of the patients[59].

SUMMARY

The incidence of prostatic cancer constitutes about 1.22% among over all malignancies reported in histopathological examination at Coimbatore medical college and hospital.

Prostatic carcinoma is found to be more common in the age group of 71-80 Years[4].

It was observed in the present study that in benign prostatic hyperplasia, AgNOR dots are large, regular and homogenously stained in the nucleus.

Whereas, in prostatic adenocarcinoma, the AgNOR dots were smaller and irregular in size and shape with giant and bizarre clusters and appear fused[69].

Of the various proliferative markers AgNOR staining using a silver compound is a simple procedure, low cost, easy to use and for early diagnosis and subsequent treatment of the prostatic carcinoma.

It also helps in identifying the aggressiveness of the tumour[5].

The present study also highlights the inverse relationship between size and number of AgNORs.

In prostatic adenocarcinoma, the number of AgNOR dots increase whereas the size become smaller[71].

Also in malignancy, individual AgNORs were obscured and appear fused.

These dots are seen attached to the periphery of the nucleus.

AgNOR is simple method which can be used adjunct to haematoxylin and eosin staining to diagnose prostatic cancer in doubtful cases[73].

The staining procedure is also simple and cost effective but needs lots of dedication and meticulous bench work to get accurate results.

CONCLUSION

In conclusion, AgNORs proves to be a simple, inexpensive, reliable method among other proliferative markers and can be used along with haematoxylin and eosin stained slides to distinguish between prostatic adenocarcinoma and benign prostatic lesions in doubtful cases and also in highlighting the aggressiveness/ prognosis of the cancer.

ANNEXURE I

PROFORMA

Coimbatore medical college

Department of pathology

Coimbatore

Particulars of the patient:

Name: Age:

Ward: Ip no./Op no.

Address: Occupation:

Presenting complaints:

Dysuria

Burning Micturition

Drippling of urine

Duration of presenting complaints

Past history:

History of previous surgeries

History of chemotherapy/ Radiotherapy

Family history

Personal history:

Diet

General examination:

Nourishment: Pallor:

Built: Jaundice:

Conscious: Cyanosis:

Clubbing:

PR: RR: BP: Febrile/ afebrile:

Lymphadenopathy: Edema:

Digital Rectal Examination:

Clinical Diagnosis:

Investigations:

Serum PSA levels:

USG Report

FINAL REPORT:

Specimen : Biopsy/ TURP

HPE Diagnosis : BPH/ BCH/ ASAP/ PIN/ PC

Gleason grading :

AgNOR stain :

Final Diagnosis :

ANNEXURE II

HAEMATOXYLIN AND EOSIN STAINING METHOD

REAGENTS USED

1. Haematoxylin solution- Erhlich'shaematoxylin
2. Eosin Y 1% solution
3. Acid alcohol 1% solution.

PROCEDURE

1. Deparaffinize sections
2. Immerse the sections in xylene for 30 minutes.
3. Place the sections in isopropyl alcohol in 15 minutes
4. Wash in running tap water
5. Stain in Erhlich'shaematoxylin for 10 to 15 minutes.
6. Differentiation is done with 1% acid alcohol two to three dips.
7. Blueing is carried out for 10 minutes.
8. Counterstain with eosin 1% solution 2 to 3 dips.
9. Running tap water wash.
10. Air dry the sections
11. Mount with DPX

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MASTER CHART

S. NO.	HP NO.	AGE	M	IP NO	TYPE OF BIOPSY	DIAGNOSIS	GLEASONSCORE	GLEASON GRADE	AgNOR COUNT	MEAN AgNOR COUNT	STANDARD DEVIATION
1	3475	62	M	26746	TURP	BPH	_	_	1.7		
2	3944B	63	M	25768	TURP	BPH	_	_	1.8		
3	1594A	70	M	27792	TURP	BPH	_	_	1.6		
4	1336B	62	M	26088	TURP	BPH	_	_	1.8		
5	2483	57	M	51834	TURP	BPH	_	_	1.2		
6	2479	75	M	50863	TURP	BPH	_	_	1.4		
7	2670	67	M	811631	NEEDLE BIOPSY	BPH	_	_	1.6		
8	2834	65	M	85552	TURP	BPH	_	_	1.4		
9	3944	63	M	67855	TURP	BPH	_	_	1.2		
10	1594	70	M	27792	TURP	BPH	_	_	1.6		
11	827	66	M	15898	TURP	BPH	_	_	1.8		
12	1359	64	M	26084	TURP	BPH	_	_	2	1.51	0.21
13	2614	73	M	80897	TURP	BPH	_	_	1.6		
14	1917	62	M	45077	TURP	BPH	_	_	1.4		
15	1643	67	M	35818	TURP	BPH	_	_	1.2		

S. NO.	HP NO.	AGE	M	IP NO	TYPE OF BIOPSY	DIAGNOSIS	GLEASONSCORE	GLEASON GRADE	AgNOR COUNT	MEAN AgNOR COUNT	STANDARD DEVIATION
16	2611	57	M	81115	NEEDLE BIOPSY	BPH	_	_	1.6		
17	2562	62	M	48845	TURP	BPH	_	_	1.4		
18	2382	59	M	48543	TURP	BPH	_	_	1.4		
19	2415	76	M	49756	TURP	BPH	_	_	1.2		
20	1911	68	M	37172	TURP	BPH	_	_	1.4		
21	1153	56	M	16996	TURP	BPH	_	_	1.4		
22	2560	71	M	79923	TURP	BPH	_	_	1.6		
23	776	50	M	11946	TURP	BPH	_	_	1.6		
24	4228	58	M	77730	NEEDLE BIOPSY	PIN	_	_	2.8	2.8	
25	3806	60	M	71599	TURP	PC	4+4	8	5.32		
26	4101	77	M	78376	NEEDLE BIOPSY	PC	4+3	7	5.2		
27	1641	70	M	31198	NEEDLE BIOPSY	PC	4+4	8	5.32		
28	4227	80	M	81315	NEEDLE BIOPSY	PC	4+3	7	5.19		
29	1088	85	M	15898	NEEDLE BIOPSY	PC	5+4	9	5.36	5.22	0.14
30	2482	75	M	50915	NEEDLE BIOPSY	PC	4+3	7	4.88		
31	1087	71	M	20766	NEEDLE BIOPSY	PC	4+4	8	5.3		
32	2755	65	M	83222	TURP	PC	4+4	8	5.21		
33	2613	69	M	81171	NEEDLE BIOPSY	PC	4+4	8	5.21		

KEY TO MASTER CHART

BPH	-	Benign Prostatic hyperplasia
PIN	-	Prostatic Intraepithelial Neoplasia
PC	-	Prostatic carcinoma
AgNOR	-	Arygyrophilic nucleolar organizer regions
HPE	-	Histopathological examination
H&E	-	Haematoxylin and eosin